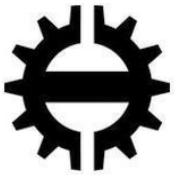

TAMPERE UNIVERSITY OF TECHNOLOGY
Master's Degree Programme
in Science and Bioengineering

BASNYAT, PABITRA

Evaluation of Toxicity of Pharmaceuticals to the Activated Sludge Treatment Plant

Master of Science Thesis

Subject approved in the Faculty Council meeting of the

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Supervisor: Professor Tuula Tuhkanen

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ABSTRACT

Different toxic pharmaceutical compounds can affect the efficiency of the Biological wastewater treatment plant. Inorder to increase the efficiency of the treatment plant, it is very important to screen these pharmaceuticals for their toxicity towards the activated sludge. Activated sludge is the main component of Biological wastewater treatment system. Therefore, in this work, 50 pharmaceuticals have been tested for their toxicity to the activated sludge. This Masteral Thesis work was done with the support of a pharmaceutical company called Universal Corporation. Pharmaceuticals have been obtained from this Corporation which is located in Nairobi, Kenya. Pharmaceutical industry manufactures many drugs like anti-HIV, anti- malarial, anti-inflammatory, lipid regulators, antibiotics, contraceptives, beta blockers and tranquilizers whose level should be minimum or they should be absent in the treated effluents from the treatment plant. OUR (Oxygen Uptake Rate) method has been used in this process for detecting the toxicity of these chemicals. This is the major principle of this method for the measurement of toxicity of chemicals. The laboratory work was done in the Tampere University of Technology. For this work, OUR (Oxygen Uptake Rate) measurement technique is applied for the toxicity test.The activated sludge was obtained from the Tampere. Oxygen meter WTW Multiline P4 with Oxygen probe CellOx325 device was used to measure the oxygen consumption rate of the activated sludge. The graphs were plotted for all the measurements and the MLSS and MLVSS values were also calculated. In the end, inhibition percentage was calculated for all the tests and EC50 concentration was calculated for the toxic pharmaceuticals. Out of 50 most popular pharmaceuticals, 11 pharmcaeuticals showed the significant inhibition percentage to the activated sludge. Aspirin, Cetrimide, Cetrizine, Chlorpheniramine Maleate, Caffeine Anhydrous, Ephedrine Hcl, Levamisole Hcl, Quinine Dihydrochloride, Diclofenac Sodium and Camphor were found toxic. The EC50 value for Diclofenace Sodium was found 23.7 mg/l. These drugs, due to its toxicity affect the efficiency of the WWTP.

Preface

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Tampere, May 2011

Pabitra Basnyat

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Abbreviations

AOP	Advanced Oxidation Process
AS	Activated Sludge
ATC	Anatomical Therapeutic Dose
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine Triohosphate
ATU	Allylthiourea
BioMnOx	Biologically Produced Manganese Oxides
BNR	Biological Nutrient Removal
BOD	Biological Oxygen Demand
CASP	Conventional Activated Sludge Plant
CO_2	Carbondioxide
COD	Chemical Oxygen Demand
DDD	Defined Daily Dose
DO	Dissolved Oxygen
DSVI	Diluted Sludge Volume Index
EC	Effective Concentration
ED	Effective Dose
EPA	Environmental Protection Agency
EU	European Union
HRT	Hydraulic Retention Time
H_2O_2	Hydrogen Peroxide
H_2S	Hydrogen Sulfide
IC	Inhibition Concentration
IIM	Insoluble Inorganic Matter

IOM	Insoluble Organic Matter
ISO	International Organization for Standardization
LAS	Linear sodium Dodecyl Bencene Sulfonate
LD	Lethal Dose
MBR	Membrane Bioreactor
MF	Micro Filtration
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NSAID	Non-Steroidal anti-inflammatory drug
OECD	Organization for Economic Co-operation and Development
OH	Hydroxyl Radical
OUR	Oxygen Uptake Rate
PhACs	Pharmaceutically Active Compounds
PPCP	Pharmaceuticals and Personal Care Products
RE	Removal Efficiency
RO	Reverse Osmosis
R&D	Research and Development
SRT	Sludge Retention Time
SF	Sand Filtration
SIM	Soluble Inorganic Matter
SOM	Soluble Organic Matter
SOUR	Specific Oxygen Uptake Rate
STP	Sewage Treatment Plant
SV	Sludge Volume
SVI	Sludge Volume Index
UCL	Universal Corporation Limited

UF	Ultra Filtration
UV	Ultra Violet
USGS	United States Geological Survey
VFA	Volatile Fatty Acids
VOC	Volatile Organic Compounds
VSC	Volatile Sulfur Compounds
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

INTRODUCTION

The major sources of wastewater are human sewage and industrial effluents. Untreated wastewater, if discharged directly to the receiving water bodies in the environment can causes water borne diseases. So the biological waste water treatment method was established in the early years of twentieth century. Biological wastewater treatment method has been applied worldwide these days. It involves the high concentration of bacteria in the tanks and they remove small organic carbon molecules by eating them. Consequently, as the bacteria grow more, the water will be cleansed and the treated water is generally discharged to receiving water bodies such as river or the sea. Different chemicals which are toxic can produce a toxic shock that kills the bacteria in the wastewater treatment plant. As a result, plant may pass untreated effluent directly to the environment. [1]

Presence of pharmaceuticals in wastewater treatment plant and the environment have caught attention during the last decade. A wide variety of pharmaceuticals (e.g. anti- inflammatory drugs, lipid regulators, antibiotics, contraceptives, beta blockers and tranquilizers) have been detected in the different water samples like river water, ground water, wastewater and drinking water [2]. Drugs that are prescribed in the hospitals and pharmacies are excreted in the faces and urine are transferred to sewage. These drugs which are untreated in water treatment remain in the discharged water. There is an increase concern about the influence of these drugs on aquatic organisms and humans because of the formation of tolerance different pathogenic bacteria to anti-microbial drugs. Inhibition of oxygen uptake rate (OUR) by 50 % is calculated to know the concentration of the toxic chemical substances. An activated sludge is used in this test to know the effect of chemicals on it. Analyzing factors affecting the elimination of pharmaceuticals by activated sludge method is very important in the wastewater treatment. [3]

This thesis consists of literature review and experimental part. The aim of the literature review is to find out what are the major inhibitory compounds in the effluent that reduces the efficiency of biological wastewater treatment system. This gives more information on what are the major group of toxic chemicals, comparison of the toxicity of different chemicals and the calculation of the EC50% of each chemical that proved to be toxic. Literature review further describes the outline of waste water treatment system, different methods of toxicity tests, application of oxygen uptake rate (OUR) test. The main aim of the thesis is to determine the inhibitory concentration of the toxic pharmaceuticals towards the activated sludge (AS). Uptake of oxygen by

microorganisms in the activated sludge is determined in the unit values. Toxic chemicals decrease the oxygen consumption rate by the microorganism and chemicals that helps in the increase in uptake rate can said to be as substrate for the microorganism. The obtained inhibitory concentration of the chemicals are compared with the standard values of most toxic chemicals such as EC50 values of 3, 5. Dichlorophenol, Diclofenac, Carbamazepine etc. There were already many researches done in the field of toxicity tests. This thesis is limited to the detection of the inhibitory concentration of the chemicals with the help of Oxygen Uptake Rate of the microorganism using activated sludge following standard Oxygen Uptake Rate procedure. Detecting and removing the most toxic chemicals in the biological wastewater treated effluent is the main purpose of this study.

A useful tool applied in this work for measuring the toxicity of the pharmaceuticals is a respirometric method called Oxygen Uptake Rate (OUR). An activated sludge was sampled at the treatment plant in Tampere and it was transported to the laboratory. It was then put for aeration throughout the using period. This aeration helps to degrade any organic matter formed due to hydrolysis in the sludge during transportation. It also helps to make the sludge homogenous by breaking down the bulky portions of the sludge. The Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS) were measured in order to see the mass of the suspended and volatile solids. Nutrient Solution was added continuously in each batch of the test. The test was run with Nutrient blank, chemical blank, sludge controls and the sludge mixed with the pharmaceuticals. For each step, DO (Dissolved Oxygen) was measured with oxygen probe for 10 minutes. Different concentrations of the chemical were tested and compared with sludge controls inorder to see the oxygen uptake rate of the sludge. With the help of these DO measurements, the specific oxygen uptake rates were measured and compared to find out if the chemical is inhibitory.

Background

The aim of this work is to detect the toxicity of the active pharmaceuticals in the treatment plant. The active pharmaceuticals are the samples that are going to be used to detect the toxicity to the activated sludge. The samples are obtained from the Universal Corporation Ltd (UCL), which is a pharmaceutical company located in the industrial area of Nairobi, Kenya. The activated sludge was obtained from the Tampere Wastewater treatment plant. Oxygen Uptake Rate (OUR) method is used for measuring the toxicity of the pharmaceuticals in the effluent. 50 different chemicals will be tested for its toxicity. Effective concentration 50% will be calculated for each toxic compound.

2. Literature review

2.1. Wastewater treatment and its component

The main aim of wastewater treatment is to remove pollutants which can harm the aquatic environment after it is discharged into it. Many oxygen demanding pollutants are organic compounds. During wastewater treatment, there are different unit operations which form a process train and they are divided depending upon fundamental mechanisms. These mechanisms include physical, chemical and biochemical basis. Physical operations include such as sedimentation which is based on the idea of physics. Chemical law defined precipitation process. Biochemical processes include living microorganisms which destroy or transform chemicals through enzymatically catalyzed reactions [4]. Increasing discharge has pressurized for the process optimization and control of the wastewater treatment plant performance .There are different methods of evaluation and regulation of the process performance in which OUR (oxygen uptake rate) is a useful tool. Organic material in wastewater is removed for reducing oxygen consuming substances in the recipient. This process is performed by bacteria as wastewater treatment plant (WWTP). The biomass in activated sludge consists of different types of bacteria. The heterotrophic bacteria in combined with other microorganisms are responsible for the degradation of main organic material. [5]

Wastewater treatment can be divided into three main stages. The removal of insoluble matters like grit, grease and scum from water by screening and sedimentation is called primary treatment of wastewater. Secondary wastewater treatment is applied to removal of soluble organic matters which are oxygen demanding, mostly by the action of microorganisms i.e. bacteria. Further removal of suspended solids and dissolved organic or inorganic material in the effluent from secondary wastewater treatment is called tertiary wastewater treatment [6].

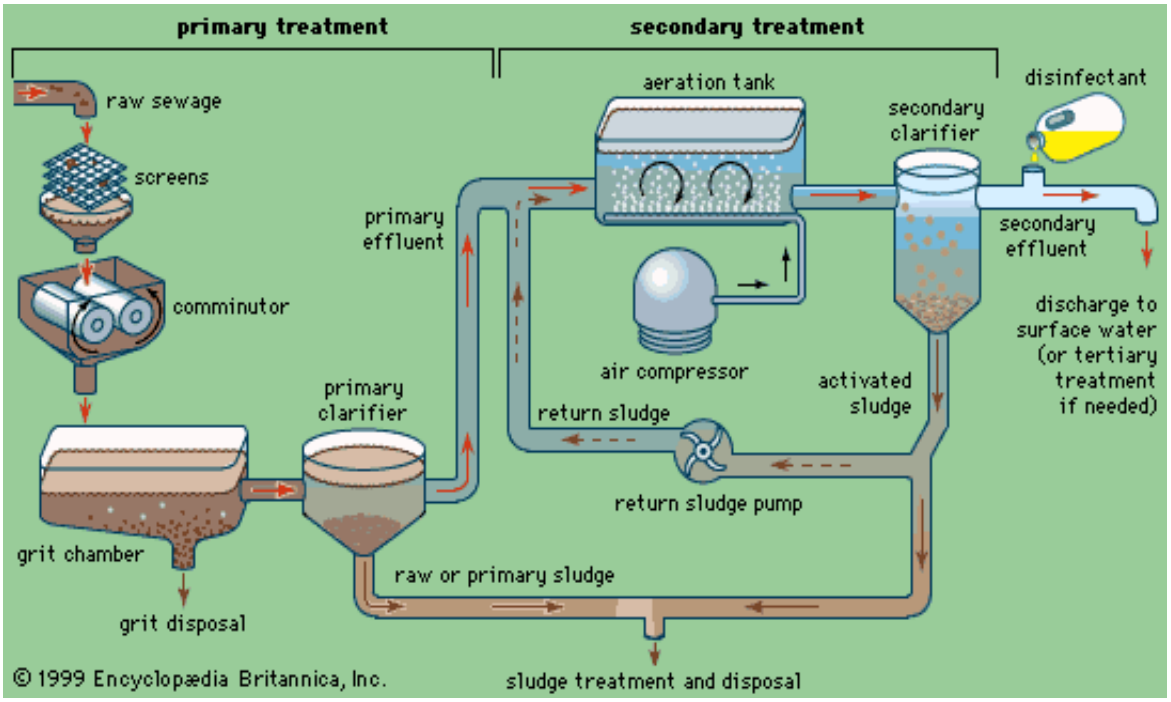


Figure 2.1. Wastewater treatment process by activated sludge method. [7]

In figure 2.1, primary and secondary treatment of raw sewage is shown with different stages in different compartments. Starting from screening till purification, different processes are shown in which formation of primary sludge, sludge treatment and disposal, activated sludge processes are shown. Early wastewater treatment systems were designed to remove organic matter. They are designed sometimes to oxidize ammonia nitrogen to nitrate nitrogen. This has been the main aim for many wastewater treatment systems these days. Engineers are more concerned about the design of wastewater treatment system in order to construct efficient and cost effective plants. Pollutants in wastewater can be classified as soluble or insoluble on the basis of physical characteristics, biodegradable or non-biodegradable on the basis of susceptibility to alternation by microorganisms. It can be further classified as organic or inorganic on the basis of chemical properties, biogenic or anthropogenic on the basis of their origin and toxic or non-toxic on the basis of their toxicity. So the main purpose of any wastewater system is to remove those materials in an efficient and economical manner. [4]

Different biochemical operations are included in the wastewater system plant. One of the major uses of biochemical operation is the treatment of sludge. Primary sludge results from the sedimentation of the wastewater and secondary sludge is produced by biomass growth in the biochemical operation. Different biochemical operations are shown in the below typical process flow diagram Figure 2.2.

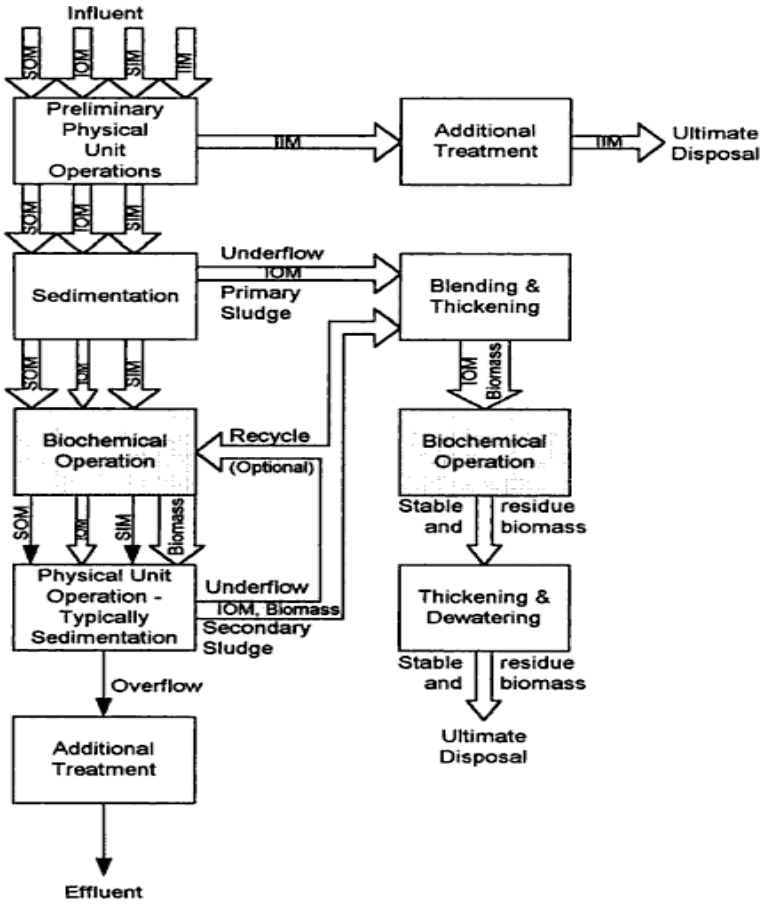


Figure 2.2.Typical process of flow diagram for a wastewater treatment system illustrating the role of the biochemical operations. SOM- soluble organic matter, IOM- insoluble organic matter, SIM- soluble inorganic matter, IIM- insoluble inorganic matter [4].

2.2 Activated sludge, parameters and efficiency

Activated sludge (AS) is the biomass which is produced in raw or settled wastewater due to the growth of the organisms in aeration tanks in the presence of Dissolved Oxygen (DO). Sludge is so called activated, because the particles are teeming with bacteria and protozoa. Activated sludge is a process in the treatment of sewage where air or oxygen is forced into sewage liquid for the development of a biological floc that reduces the organic content of the sewage. The sewage, after sufficient treatment, excess mixed liquor is discharged into the settling tanks and the supernatant is run off for the further treatment prior to discharge. This phenomenon occurs mostly in all activated sludge plants. Some part of the settled sludge is returned to the head of the aeration system and remaining sludge is further treated before it is disposed. [8] The overall function of the activated-sludge process is to remove substances which have a demand for oxygen from the system. This process is followed by the metabolic reactions (synthesis- respiration and nitrification) of the microorganisms, the separation and settling of activated sludge solids for the creation of acceptable quality of secondary

wastewater effluent and the collection and recycling of microorganisms back into the system or removal of excess microorganisms from the system. [9]

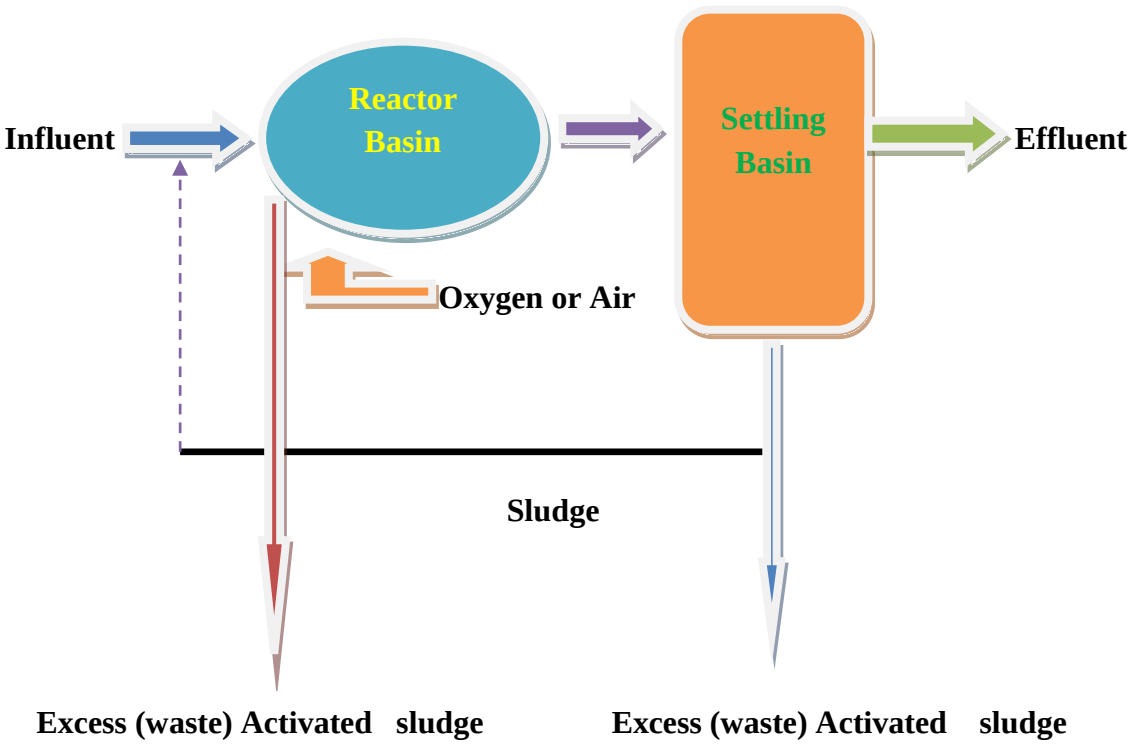


Figure 2.3. Typical activated sludge process.

Typical activated sludge process is shown in a schematic form in Figure 2.3. When an influent enters inside the reactor basin, it comes out as an effluent forming the activated sludge in the intermediate stages. There are several parameters of activated sludge used in biological wastewater treatment. Mixed Liquor Suspension Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Sludge Volume Index (SVI), Oxygen Uptake Rate (OUR) are the different parameters of activated sludge. These parameters are used to analyze the condition and quality of the sludge. Efficiency of the activated sludge process in treating wastewater can be examined by measuring the biochemical oxygen demand (BOD) of the wastewater before and after the activated sludge process. MLSS is a measure of inorganic and organic suspended materials present in the aeration basin. It is measured by filtering a known sample volume through a filter and weighing the mass difference. MLVSS is a measure of volatile suspended material present in the aeration basin. These are mainly the organic materials which are used as a measure of the concentration of microorganisms. It is measured by burning the MLSS sample in 550 degree centigrade and weighing the mass difference. Sludge Volume Index (SVI) is a measure of settling capability of activated sludge. It is an experimental result which is useful in routine process control. This is measured by letting a volume of one liter of sludge settles for 30 minutes, reading the level of sludge (sludge volume at 30 minutes, SV30) and dividing it by MLSS. This is reasonable for the sludge volume 300 ml, if the amount exceeds the sample is diluted and the same process is repeated that gives the Diluted Sludge Volume Index, DSVI. [12]

$$SVI = \frac{SV_{30}(ml/l)}{MLSS(g/l)} \quad (1)$$

Equation (1) gives the mathematical expression about how to calculate the Sludge Volume Index (SVI). SVI can be obtained by dividing SV_{30} by MLSS value. OUR (Oxygen Uptake Rate) is another important parameter of the activated sludge which is discussed later in the different chapters.

Biochemical Oxygen Demand (BOD) is a measure of oxygen usage by a water sample in a specific time. The oxygen is used by the biochemical degradation of dissolved and suspended oxidable matter in water. BOD is an approximation of easily degradable matter which is used to evaluate the water quality and water treatment efficiency. [12]

2.2.1 Biochemical aspects of Activated Sludge

Microorganisms are mainly present in the biological component of the activated sludge. There composition is 70 to 90 percent organic matter and 10 to 30 percent organic matter. Their types depend upon the chemical composition of the wastewater and the specific characteristics of the organisms in the biological community. Microorganism play an important role in the activated sludge process, they help in removing carbonaceous organic material and nitrifying ammonia in secondary influent wastewater. There are two levels of structure present in the activated sludge flocs. One is microstructure and another is macrostructure. Microstructure consists of microbial aggregation, adhesion and bioflocculation. As the meaning of bioflocculation is not clear, it is felt to be the result of bridging between extracellular microbial polymers. These polymers function as polyelectrolytes such as substances of high molecular weight like proteins which is an ionic conductor. Those extracellular microbial polymers form the envelopes like structure around the cells and group of cells. [9]

There is the production of organic compounds and inorganic compounds due to the anaerobic activity in sewer systems. Mostly, malodorous compounds like volatile organic compounds (VOCs), volatile fatty acids (VFAs), volatile sulfur compounds (VSCs) and the inorganic gases like ammonia ($NH_{3)}$, hydrogen sulfide (H_2S). Organic compounds are degraded by bacteria for obtaining carbon for cellular growth and reproduction and energy for cellular activity. Bacterial growth causes increase in the biofilm that covers sewer mains. Carbon atoms and electrons are released as chemical bonds due to the bacterial degradation of organic compounds. New cellular materials are produced from these carbon atoms and the electrons are used to obtain energy during electron movement from one protein molecule to another in an electron transport system. These electrons are removed from the cells at last. Several molecules are used

by bacteria to remove the electrons from the cells. These molecules consists of free molecular oxygen, nitrate ions (NO_3^-), nitrite ions (NO_2^-), sulfate ions ($SO_4^{=}$) and an organic compound. [13]

Activity measurements of enzymes have been done in the microorganism population in the activated sludge. The enzymatic activities of the activated sludge that come from different WWTP show a high ability of microorganisms to change polyphosphates. These activity measurements studies shows that the suitability of the activity measurement which defines the potential possibility of a microorganism population for carrying out biological phosphate removal by the activated sludge method. [14]

2.2.2 Microbiological aspects of Activated sludge

The microbes convert carbon into cell tissue and oxidized the end products which includes carbon dioxide (CO_2) and water. Along with this, a limited number of microorganisms may exist in activated sludge which gets energy by oxidizing ammonia nitrogen to nitrate. This process is called nitrification. Majority of the microorganism are present in the activated sludge. Heterotrophic bacteria are predominant which require organic compounds for their supply of carbon and energy in activated sludge. On the other hand, it consists of autotrophic bacteria that occur in proportion to concentrations of carbon and nitrogen. Aerobic and anaerobic bacteria may exist in the activated sludge; facultative species are preponderance which can live in absence r lack of DO (Dissolved Oxygen). Fungi, rotifers and protozoan are also present in the activated sludge. They may be ciliated or flagellated protozoan and amoeba is also present. These protozoans are the indicators of the activated sludge condition. Viruses of human origin are also found in raw sewage influent [9]. In Figure 2.4, different kinds of microorganisms are shown such as bacteria and parasites.

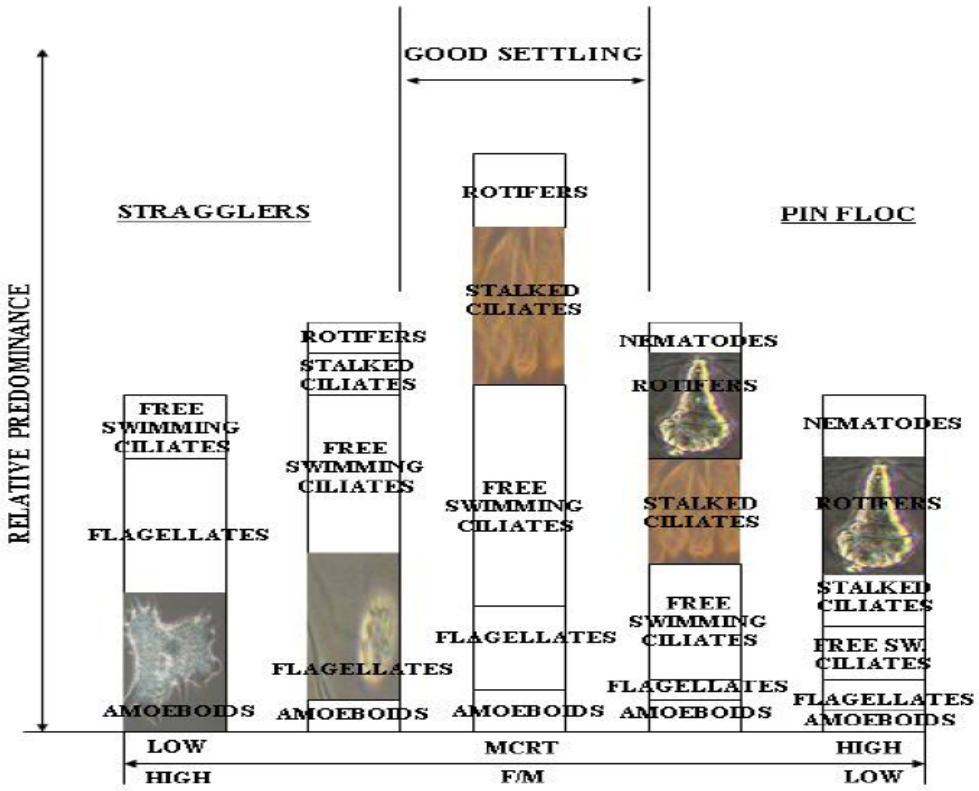


Figure 2.4. Figure showing the wastewater micro life [10].

Major nutrients in sewage are nitrogen and phosphate. In Finland the phosphate is removed chemically. Biological nitrogen removal is the important part of the activated sludge processes in Finland. Initially in the activated sludge process the biomass is ammonificated to ammonia and then it is nitrificated to nitrite and further to nitrate which forms nitrogen gas through denitrification. Another method is the Anamox reaction in which the nitrogen is removed biologically where ammonia ion and nitrite ion forms nitrogen gas. *Nitrosomonas aeuropea and Nitrobacter species* bacteria are said to be mainly responsible for nitrification. [11]

2.3. Comparative study of different types of toxicity tests

Microbial population in an activated sludge is a heterogeneous community which is in equilibrium and helps the treatment plant to be flexible with respect to plant operational changes, flow, temperature and wastewater composition variations etc. Toxic chemicals may inhibit the activity of activated sludge which causes the loading shock to the system causing in lower treatment efficiency or the breakdown of the system as well. [16]

Conventional parameters such as chemical oxygen demand and suspended solids are not useful for detecting toxic compounds that are present in the industrial wastewaters and treated wastes. Therefore, due to the toxicity in many industrial wastes causes more effect on biological wastewater treatment process. It causes adverse effects on receiving waters bodies [15]. Thus there are different techniques implied for the toxicity tests in the biological wastewater treatment process.

Microtox test

Microtox assay is based on the naturally occurring luminescent marine bacterium *Vibrio fischeri* that consists the constitutive promoter controlling the *luxCDABE* gene. Luminescence of *V. fischeri* is repressed in the presence of toxicants. It responds to the toxicants in a ‘‘lights off’’ fashion. [17]

Microtox test is an acute toxicity test. It offers the greater sensitivity, repeatability and precision. These tests are used to study the pinpoint unusual wastewaters, evaluate the toxicity reduction through activated sludge processes. It is also used in observing the effect of increased chemical addition to meet the transparency standard, and also to measure the impact of waste discharge on one particular receiving water [15]. It is a common toxicity tool for the screening of wastewaters discharged into wastewater treatment plants. In one of the study performed by Gutierrez [16], Microtox was proven of having the higher sensitivity to toxicants but it was fewer representatives of effects on activated sludge compared to respiratory technique of toxicity test. In one assay related with LAS (Linear Sodium Dodecylbencene Sulfonate), a biodegradable reference surfactant showed the toxic effect following Microtox test whereas respirometry technique performed a good biodegradability but no toxicicity effect. [16]

Daphnia magna test

Daphnia magna is an acute toxicity tests which is performed by using daphnid *Daphnia magna*. This test is based on the bioluminescence method. It is an ecotoxicity assay for ecotoxicity evaluation of wastewater. Current legislation contains various parameters for water quality control which includes ecotoxicity determination by reference test like Daphnia magna mobility test. [16]

Nitrification inhibition tests

Nitrification assays are generally performed using nitrifying activated sludge or purified nitrifying bacteria. The disappearance of ammonium-containing substrate and the appearance of nitrite or nitrate can be monitored and used to asses nitrification inhibition. It is essentially an oxidation process and requires oxygen. Nitrobacter assay was used for toxicity identification and evaluation of nitrification inhibiting substances in industrial wastewaters [17]. Microtox and nitrification inhibition tests are too sensitive for screening toxic chemicals. [16, 17]

OUR (Oxygen Uptake Rate)

OUR measurement is a useful test for the evaluation and regulation of the process performance. It is mainly used as the toxicity test in biological wastewater treatment plant. By determining the oxygen consumption rate during a limited period of time, the oxygen uptake rate can be calculated. The OUR measurements are not very difficult to perform practically but it needs the more effort for interpreting the results into useful information. [5]

Oxygen Uptake Rate is a respirometric test. It measures the toxicity effects of different chemicals on activated sludges. Ubey [16] has explained the respirometric analysis of synthetic domestic sewage along with textile, dairy, meat processing, tannery and confectionary wastewaters. This was done for the experimental assessment of the readily biodegradable COD which includes the accuracy and reliability of the method. [16]

Respirometry techniques characterize the toxicity of the effluents better than other techniques. Activated sludge respirometric tests are more direct method for assessing sludge activity and toxicity to sludge comparing to bioluminescence methods of toxicity tests. Different activated sludge respirometric techniques are well established and several standardized tests have been existed for a long time. For example, Organization for economic co-operation and development (OECD), 1984, Environmental Protection Agency (EPA) 1996, International Organization for Standardization (ISO) 1986. [20]

Table 2.1 is shown inorder to see the criteria for influent wastewater toxicity monitoring. These comparisons of the different assays and devices can provide the useful information. It is shown that no single method has fulfilled all the criteria. Criterion 1 shows the bioassay based system that indicates overall toxicity without analyzing individual components of wastewater samples. Likewise different criterion shows different information in Table 2.1.

Table2.1. Summary evaluation of methods [a] for assessing wastewater toxicity to activated sludge [b]. [17]

Number	Criterion	Biolumines cence methods	Respirometric Methods [c]	Respirometric Methods(nitrificati on/ denitrification)
1	Overall toxicity	+	+	+
2	Predict process upset	?	?	?
3	Identify source	-	-	-
4	Rapid response	+	-	-
5	Anaerobic/aerobic	-	+	+
6	Concentration-response	+	+	+
7	Depict corrective action	?	?	?
8	Ease to operate/maintain	-	-	-
9	Accurate interpretation	+	+	+
10	Function in complex matrix	?	+	+
11	Cost effective	?	?	?
12	Detect false positive/negatives	?	?	?
13	Active/sleep mode switching	+	+	+
14	Commercializable	?	+	+

[a]Only methods reviewed in this manuscript were included in the evaluation.

[b]The same sumbols were as in Love and Bott (2000): ‘‘ +’’ indicates that method meets the criteria, ‘‘ -’’ indicates that method does not meet the critera, and ‘‘ ?’’ indicates further investigation is necessary to assign ‘‘ +’’ or ‘‘ -’’.

[c]Method excludes nitrification/denitrification inhibition assesment.

2.4. Oxygen Uptake Rate (OUR) measurement for application at waste water treatment plants.

The toxic compounds can inhibit growth or respiration in heterotrophic bacteria present in the wastewater. There are different ways by which the inhibitory compounds can act. They can be inhibitory to their own biodegradation or they may influence the rate of biodegradation of other compounds. Respiration involves the breakdown of simple organic carbon molecules. The end product is carbon dioxide and water. The nitrifying bacteria, chiefly Nitrosomonas and Nitrobacter, oxidize ammonia to nitrate and then to nitrite [18]. The steps are:

$$NH_3 + O_2 \xrightarrow[Nitrosomonas]{} NO_2 + O_2 \xrightarrow[Nitrobacter]{} NO_3 \quad (2)$$

Equation (2) shows the oxidation of ammonia to nitrate and then to nitrate. When growth is inhibited, the energy requirements will decrease; as a result there will be decrease in the respiration rate. Therefore less carbon is required for the respiration which results in decrease in biodegradation. Therefore, when there is toxicity, there will be inhibition in both the respiration rate and the biodegradation rate. [19]

2.4.1 Respirometric techniques

Respirometry is the process of measuring the biological oxygen uptake rate in aerobic conditions and interpreting the result. It is also the measurement of biogas generation rate in anaerobic environment. Respirometric methods are associated with the activated sludge in the wastewater. [21, 22]

The first application of respirometric technique was used for measuring the oxidation of wastewaters. It was reported in 1924 by Otto Heinrich Warburg (1883-1970). Manometry was the respirometry technique. Many researchers have developed different kinds of respirometric measuring techniques on manometric, electrolytic or direct measurement of oxygen consumption based on Warburg's respirometer. Modern invention of automation and instrumentation has widened the applications of respirometer in optimizing and operation of wastewater treatment systems and waste management and biodegradation researches. [23, 24, 25]

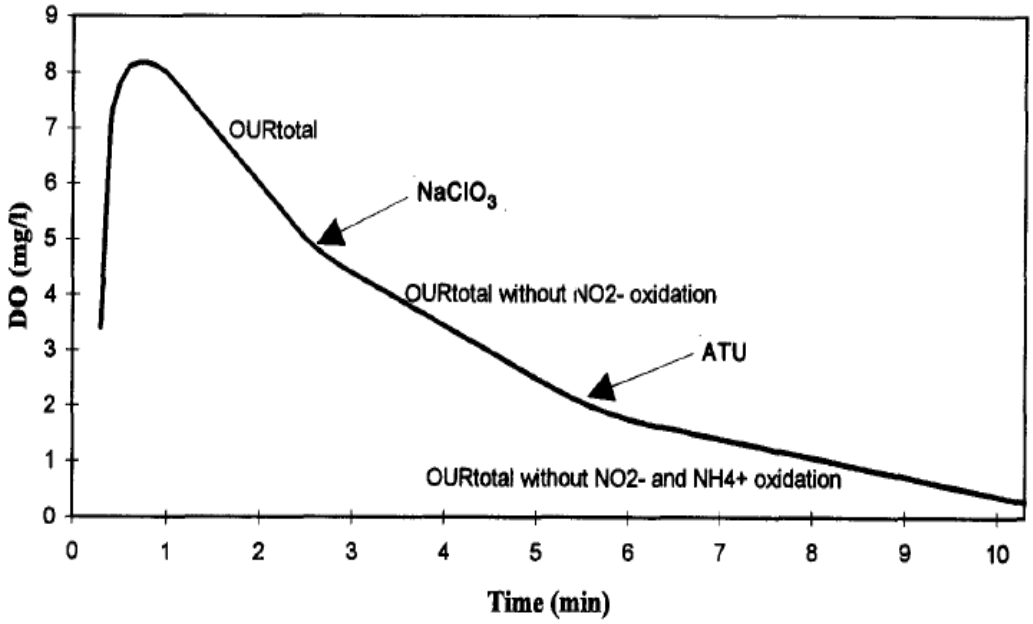


Figure 2.5. Schematic representation of a typical oxygen uptake profile recorded with the developed nitrification activity determination method. [26]

Respirometry is a useful mechanism for identification of the compounds which may potentially have an adverse effect on a wastewater treatment plant. The figure 2.5 shows the activity measurements result in oxygen uptake profiles. This shows the rapid, simple and robust method for the determination of NH_4^+-N and NO_2^- -N oxidation rates by measuring the OUR of mixed liquor samples before and after adding the selective nitrification inhibitors. ATU (Allylthiourea) and $NAClO_3$ (Sodium Chlorate) are the selective inhibitors of Nitrosomonas Nitrobacter species. This method is validated on a SBR (Sequential Batch Reactor). [26]

2.4.2 Oxygen Uptake Rate (OUR) Principle

During the respiration of microorganisms, bacteria convert the energy from the organic substrate to high energy compound ATP. In the substrate, the electrons are removed by oxidation. These electrons are transferred along an electron transport chain to the terminal electron acceptor i.e. oxygen in the aerobic respiration. In this process the ATP is generated, so the process is called oxidative phosphorylation. These ATPs are used by bacteria as an energy source for the synthesis of different molecular compounds that are necessary for cell growth and reproduction. Nearly half of the substrate molecules are converted into new biomass in activated sludge. [22]

Oxygen uptake Rate is directly related to the dissolved oxygen (DO). DO concentration is generally used to control activated sludge process with the help of

information about growth and substrate utilization in addition with the respiration rate-based control comparison. [22]

Oxygen Uptake Rate is the uptake of oxygen by activated sludge microorganisms per unit volume of sludge, in unit time. In the presence of a suitable, easily biodegradable substrate, activated sludge will consume oxygen rapidly. Addition of toxic concentration of a test material can result in the decrease in oxygen consumption rate as shown in Figure 2.7. The rate is measured with an oxygen electrode. The percentage inhibition of the oxygen consumption can be estimated by comparing a control mixture which doesn't contain a test material [20]. EC50, EC20 and EC10 values are used in respiration inhibition and nitrification inhibition tests for representing the concentration of wastewater (%) which produces 50%, 20% and 10% inhibition of the oxygen uptake rate in comparison to the control sample. [28]

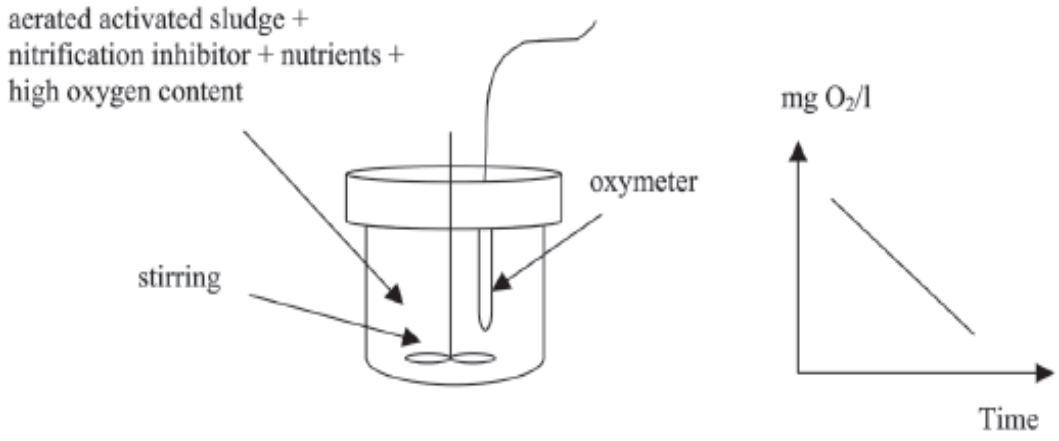


Figure 2.6. Illustration of the principle of the OUR measurements. [5]

Oxygen uptake rate measurements provides the information related to treatment plant performance, wastewater characteristics, degradability of special concentrated streams and also the parameters required for mathematical models for the prediction of possible optimizations of a treatment plant.[5]

The Specific Oxygen Uptake Rate (SOUR) is known as the oxygen consumption or respiration rate. It is defined as the milligram of oxygen consumed per gram of volatile suspended solids (VSS) per hour. This is a quick test which has many advantages. It rapidly measures the influent organic load and biodegradability which is the indication of the presence of toxic or inhibitory wastes. It also provides the degree of stability and condition of a sample, and calculation of oxygen demand rates at various points in the aeration basin. This test was originally developed as a plant control parameter. [27]

2.4.3 OUR Method

Aerated activated sludge containing necessary nutrients and nitrification inhibitor is used for OUR measurement. Nitrification inhibitor is used to eliminate the oxygen consumption due to nitrification. There will be decrease in oxygen concentration for some minutes are noticed. The relationship between the decrease in oxygen concentration and time is normally seen to be linear as shown in Figure 2.6.

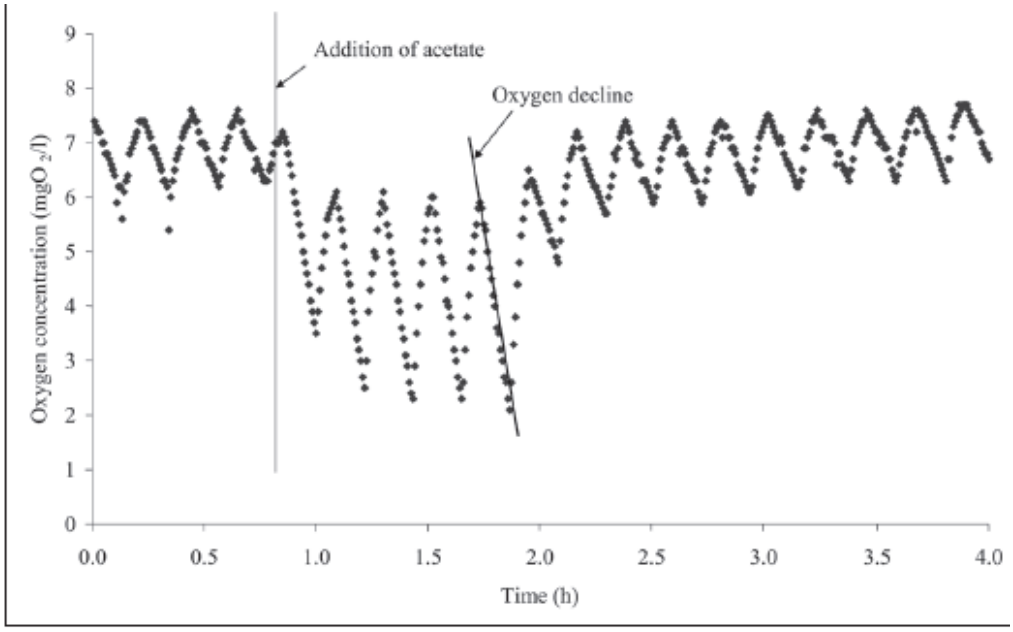


Figure 2.7. A plot of the Oxygen concentration during repeated aeration in activated sludge with addition of acetate after 0.8 h. [5].

The OUR can be determined by calculations of the slope of the curve. Specific oxygen uptake rate is obtained if the oxygen uptake rate is associated with the volatile suspended solids (VSS). OUR can be followed longer by alternating the aeration of the sludge in intervals. OUR equipment is also called respirometer consists of a basic system which needs manual data collection and calculation. But more advanced commercial systems which are computerized and calculations can be done automatically have been used these days. However the types and the operating mechanisms are different. The essential components that includes are a test chamber for adding aerated mixed liquor, a stirring mechanism, a dissolved oxygen probe and a dissolved oxygen analyzer as shown in Figure 2.8. [29]

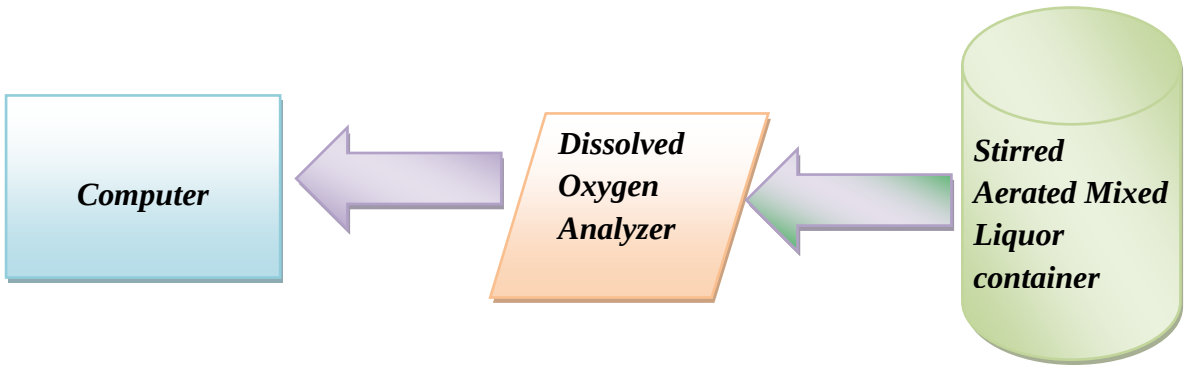


Figure 2.8 Schematic diagram for the test equipment for OUR determination.

In the laboratory OUR can be measured by using a closed container and measurement of dissolved oxygen is taken by using an oxygen electrode and meter, when there is no mass transfer with the environment. [22]

2.4.4. Factors affecting Oxygen Uptake Rate

Different experimental conditions should be applied while performing the OUR test. Alterations in these factors may affect the OUR test results. Different factors that affect the OUR measurements are carbon source, pH, Nitrification inhibitor and Temperature.

OUR is different with the different kinds of organic substrate so it is very important to use the same substrate. Same substrate should be used to compare the capacity of different activated sludge. Generally, acetate is used as a reference substrate because it is very easily degradable organic matter for heterotrophic bacteria. Therefore carbon source is one important factor in OUR test. Another factor is pH. The aerobic degradation of organic matter depends upon the pH 6 to 8. pH will slightly increase because CO_2 is produced during oxygen respiration. Normally, there is no need of adjustments for pH stabilization. Temperature is another factor in OUR measurements. Generally OUR increases with the increase in the temperature so it is very important to keep the temperature constant in the whole experimental period. Generally the laboratories experiments are performed in 20˚C. [5]

Another factor is the nitrification inhibitor. It is used only when the sludge has been obtained from the nitrifying treatment plant. In such Sludge, the oxygen consumption depends upon the oxygen used for nitrification instead of oxidation of organic matter. So nitrification inhibitor is used to prevent nitrification during measurement of organic degradation. Allylthiourea (ATU) is generally used as nitrification inhibitor which inhibits the conversion of ammonia to nitrite. Approximately 12 mg/l is used for OUR test measurement. It has been shown that use of 10 mg/l of ATU has impact on the endogenous respiration of the sludge that finally results in the low OUR results. Therefore the amount may be different for different types of applications followed. [5]

Mixing rate and the concentration of oxygen demanding material in the sample are the major factors that affect oxygen uptake measurements with the electrolytic respirometer these problems can be avoided by using simple procedures like increased mixing rate or enrichment of the oxygen in the air which is in contact with the sample. [34]

2.4.5 OUR Applications

Oxygen Uptake Rate (OUR) measurements were initially used in wastewater treatment plants for monitoring biological activity of conventional BOD removal systems. The most common use of OUR measurements by plant operators was to determine viability of the organisms. Oxygen Uptake Rate (OUR) testing is a simple, readily available, and familiar tool which can provide more information like monitoring, optimization and troubleshooting BNR (Biological Nutrient Removal) systems. [29]

The respirometry test is well established these days and it is being used in both research and at wastewater treatment plants. Interpretation of the results is complex for this type of tests. OUR measurements are used for the characterization of wastewater streams in both the batch tests and online respirometry. This test is used in different wastewater streams such as municipal wastewater, concentrated organic streams from industries and internal recirculation streams from different parts of the treatment plant. OUR is applied in the variations in organic load/treatability in which it is useful for the plant operator to control and manage the plant in a more optimal manner. [5]

OUR tests are explained to be more useful for characterization of industrial wastewater. It is important in wastewater treatment plants for continuous measurements and used as forward treatment strategy and also for plant performance control. [30]

Graph 2.9 below shows that with a 100% concentration of the wastewater, the respiration rate is inhibited by 46% relative to the control. The EC50 is >100mg/l, EC20 is 60mg/l and EC10 is 40mg/l. These values show that the wastewater is toxic.

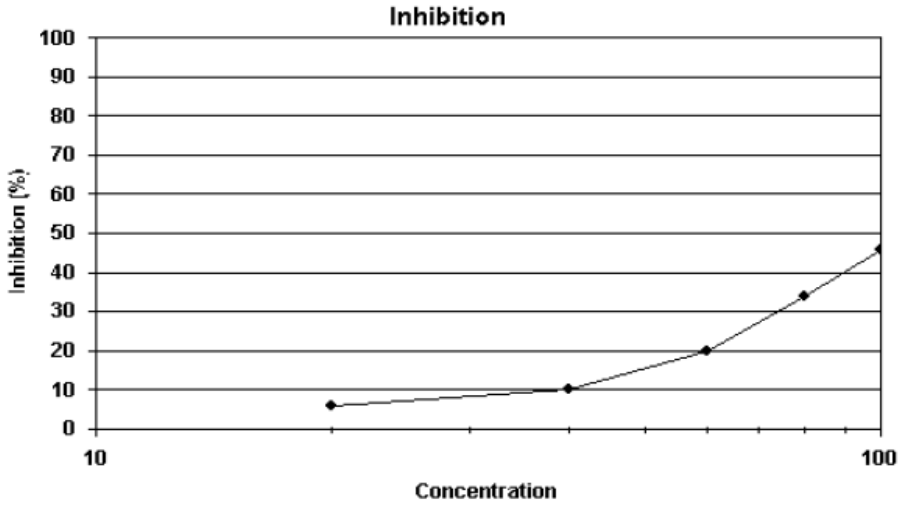


Figure 2.9. The plot of respiration inhibition vs wastewater concentration [28].

The most important application of OUR is the toxicity test and inhibition measurement of the wastewater streams. In Figure 2.10, the effect of adding toxic water to activated sludge is demonstrated. Addition of acetate results in the rapid increase in the OUR but the biological treated leachate water only shows a smaller increase when mixed to the activated sludge. The toxic wastewater results in the decrease in the OUR compared to the endogenous respiration level which indicate the decay or inhibition of the microorganisms in the sludge. [5]

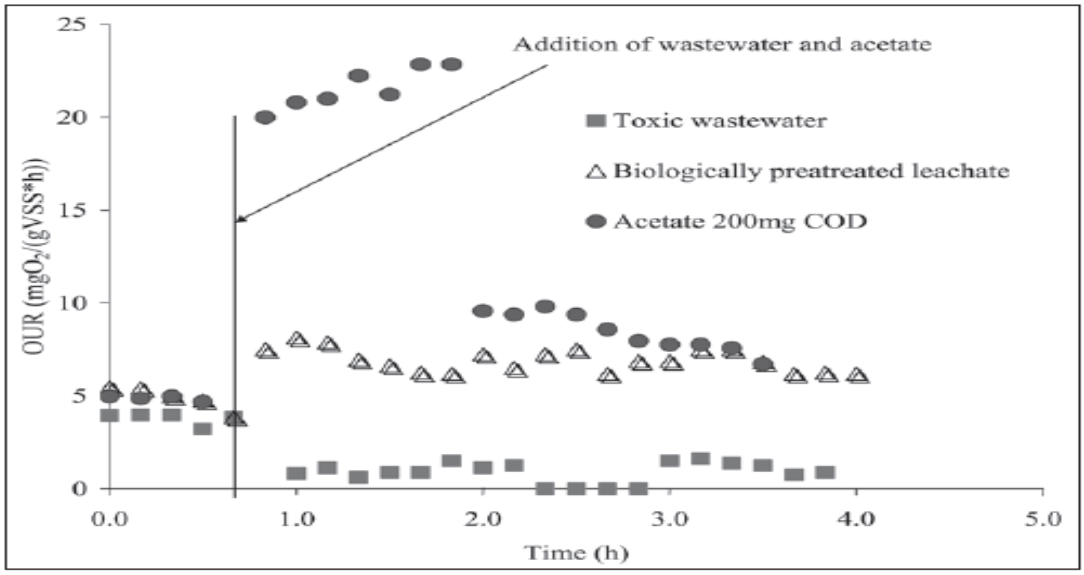


Figure 2.10.Toxicity test. Three parallel respiration tests, one with additions of acetate, one with biological pre-treated leachate and one with toxic wastewater. Sludge from the same plant with identically conditions was used in all reactors. [5]

Respirometry tests are important because the results can be received quickly. For obtaining more quantitative explanation of the toxic effect it is often used in

combination with EC50 measurement. EC50 represents the concentration of a compound where 50% of its maximal effect on the tested organism is observed. It will be more useful if the measurement is taken repeatedly at the same spot inorder to detect the changes in the result. Regular OUR tests at different places of the plant are useful in following the process performance. Continuous OUR measurement will help the plant operator for fast troubleshooting of process failures and process changes. [5]

The importance of respirometric methods is that it is possible to measure the respiration activity easily and quickly. New technologically advanced respirometers are used for the biodegradation measurements in water media and soils, for kinetic analysis and biokinetic constant determination, for modeling the wastewater treatment process, for managing toxicity, for measuring short-term BOD, nitrification capacity, activity of activated sludge, readily degradable BOD treatment capacity and aeration requirements. [21, 28]

2.4.6. EC50 and its Interpretation

The toxicity of chemicals is generally expressed in terms of dosage that gives 50% effect to the response in comparison with the control. The effect can be more or less in response. This is called EC50 or Effective Concentration 50. It is also said to be Effective Dose 50 (ED50) or RD50 for dosage causing 50% inhibition or reduction .It is referred to as LD50 in animals, the dosage lethal to 50% of the subjects. The EC50 is generally estimated by fitting a log-logistic curve to the data. The model is a sigmoidal relation on a logarithmic scale rather than linear relation. The logistic model can be used to dichotomous data like survival or death and to continuous data such as weight or biomass, and can be expressed in terms of length for growth [31]. EC50 is a function of chemical concentration to construct a frequency distribution of the affected species. This is called as species sensitivity distribution (SSD). [32]

A standard dose-response curve can be explained by four parameters. The baseline response (Bottom) the maximum response (Top), the slope, and the drug concentration provokes a response halfway between baseline and maximum (EC50). The definition can be simplified as the concentration of agonist which provokes a response half way between the baseline (bottom) and maximum response (Top). EC50 cannot be defined without defining the baseline and maximum response. It is easy to misunderstand the definition of EC50. [33]

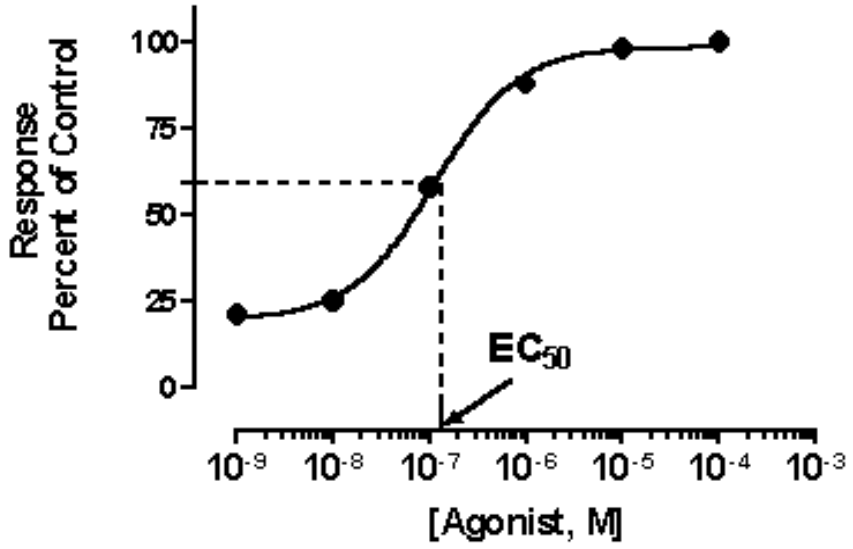


Figure 2.11 Standard dose response curve. [33]

It is defined quite simply as the concentration of agonist that provokes a response half way between the baseline (Bottom) and maximum response (Top). An Agonist is a drug which causes a response and an antagonist is a drug which doesn't cause response in itself. In Figure 2.11, response can be seen due to the agonist. It is impossible to define the EC50 until the baseline is first defined and maximum response. The baseline is about 20%, and the maximum is 100%, therefore the EC50 is the concentration of agonist which evokes a response of around 60% (half way between 20% and 100 %). EC50 shouldn't be overinterpreted. [33]

2.5 Pharmaceuticals in the wastewater

2.5.1 Pharmaceuticals

According to EU definition a pharmaceutical, or a drug or a medicinal product is defined as any substance or combination of substances presented as having properties for treating or preventing disease in human beings. It can be also defined as any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to make a medical diagnosis. Human pharmaceuticals consist of variety of chemical structures. Approximately 3000 active components are used in Europe. Classification of pharmaceuticals is very difficult and it can be classified usually on the basis of chemical structure, pharmacological activity, physiological classification and receptor interaction. Each pharmaceutical consist of an active pharmacological compound. [38]

Pharmaceuticals are biologically active compounds that are made for the treatment of different diseases. There are different methods of classifying

pharmaceuticals. The Anatomical Therapeutic Chemical (ATC) and the Defined Daily Dose (DDD) is the measuring unit which have become gold standard for international drug utilization research. The ATC/DDD system is a means for exchanging and comparing data at international, nationals and local levels. [38]

The presence and the nature of the pharmaceuticals in wastewater treatment and the environment have created the more interest during the last decade. New pharmaceuticals are exponentially added in the large array of chemical classes in the markets. [35,36]. Different types of pharmaceuticals like anti-inflammatory drugs, lipid regulators, antibiotics, contraceptives, beta-blockers and tranquilizers have been detected in different water samples from river water, groundwater, wastewater and drinking water. [35, 36, and 37]

Pharmaceuticals are generally classified as antibacterial, antiepileptics, anti-inflammatory and antirheumatics, beta-blocking agents and lipid modifying agents. Antibacterials drugs were again classified into the Fluoroquinolones. They are Ciprofloxacin, Norfloxacin and Ofloxacin, Sulfonamide and the Sulfamethoxazole. The fluoroquinolones are used for the treatment of UTI (Urinary Tract Infections), respiratory infections, gonorrohea, bacterial prostatisis, cervicitis and anthrax. Carbamazepine is the widely studied antiepileptic drug. It is also used in the treatment of neuropathic pain and manic-depressive illness. [39]

Diclofenac, Ibuprofen, Ketoprofen and Naproxen are classified as anti-inflammatory and antirheumatics. They are also called non-steroidal anti-inflammatory drugs and are used worldwide for the treatment of rheumatic musculoskeletal complaints. Diclofenac and Ketoprofen are used as a therapeutic agent in different gels and sprays for treating muscle pain. Acebutolol, atenol and metoprolol are the beta blockers used for the treatment of angina, hypertension and dysrhythmias. Bezafibrate is a lipid modifying agent used in the treatment of mixed dyslipidaemia which is a risk factor for atheromatous disease. [39]

Structure of the pharmaceutical industry includes research and development (R&D) to discover, enhance and devise reliable manufacturing processes of drugs, bulk manufacturing to produce large volumes of drug ingredients. Manufactured drugs are combined with drug ingredients in a form suitable for sale and use and marketing for promoting and selling drugs (e.g., by informing health care providers and consumers of their availability, features and proper use). There are three stages of pharmaceutical production which is shown below in Figure 2.12

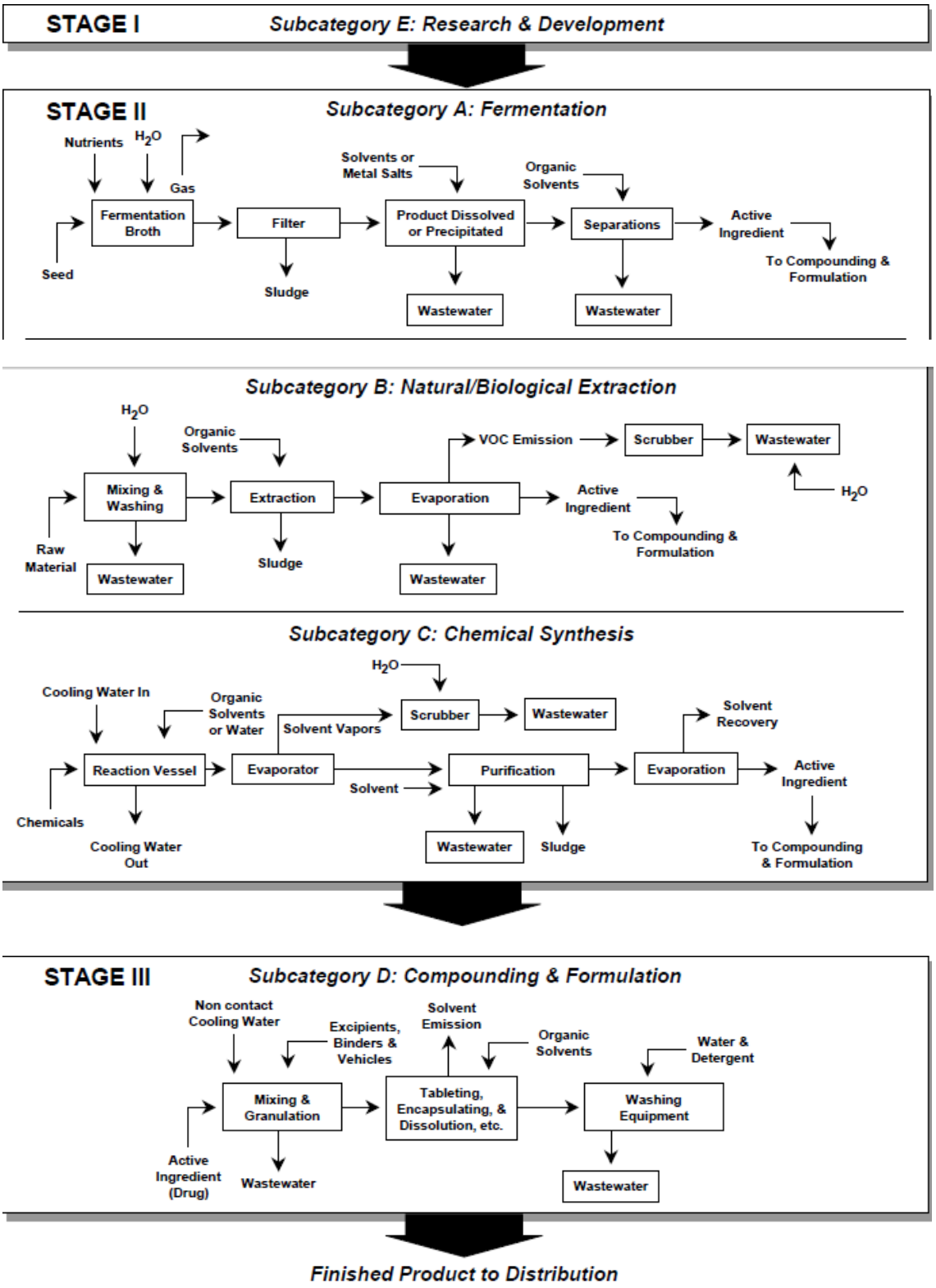


Figure 2.12.The three stages of pharmaceuticals production, Adapted from EPA, 1992. Pharmaceutical Manufacturing Industry: Revision of Effluent Guidelines. Unpublished Status Briefing. Washington, DC: U.S. EPA. [56]

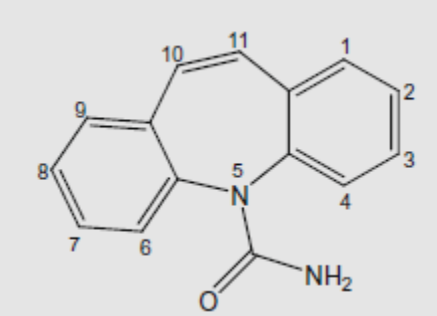
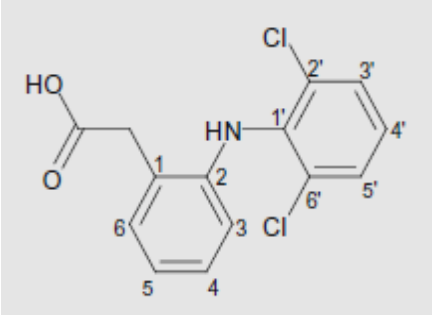
The first stage of Figure 2.12 is the Research and Development stage which helps in discovering, enhancing and devising suitable manufacturing process for

pharmaceuticals. A new drug needs many laboratories testing for years such as upto 12 years as well. The second stage is the Bulk drug manufacturing stage which converts organic and chemical substances into bulk active ingredients with the help of many conversion processes like fermentation, extraction or chemical synthesis. Stage III includes the finished pharmaceutical product formulation which applies to the combination of bulk active ingredients with other substances for the production dosage forms which are suitable to intake by human or animal. Formulation means preparing the dosage forms into tablets, capsules, liquids, parenterals and creams and ointments. Hard and soft capsules contain gelatin capsules which are filled with active ingredient. [56]

2.5.2 Major pharmaceuticals in the wastewater

The wide use of pharmaceuticals like Carbamazepine and Diclofenac has made their presence in the wastewater. All the drugs are not removed effectively in the WWTP so they can be found in the water bodies. These drugs can be found in the WWTP effluents, surface waters, and ground water and sometimes in drinking water. These drugs have been detected in Europe and Asia and America. The concentrations of carbamazepine and diclofeanc are significantly different in different countries. It is due to the diverse consumption of rates of both pharmaceuticals in those countries. In some countries the higher concentrations of these drugs may not be found due to the insufficient investigations conducted. Carbamazepine concentrations in WWTP effluents are normally hundreds of nanogram per liter but sometimes it may occur in microgram per liter varying the values between different countries. Carbamazepine has been defined as anthropogenic marker in water bodies. [40]

Table 2.2. Physical, Chemical and Pharmacological properties of Carbamazepine and Diclofeanc. [40]

	Carbamazepine (CBZ)	Diclofenac (DFC)
Pharmacology Structure, formula, CAS No. and molecular weight	 $C_{15}H_{12}N_2O$ 298-46-4 236.27 g mol^{-1}	 $C_{14}H_{11}C_{l2}NO_2$ 15307-86-5 296.16 g mol^{-1}
Usage	Analgesic, antiepileptic	Analgesic, anti-inflammatory
Water solubility	17.7 mg L^{-1} (25°C)	23.73 mg L^{-1} (25°C)
Log *P* (octanol–water)	2.45	–
Henry's Law Constant	1.09 × 10^{-5} Pa m^3 mol^{-1} (25°C)	4.79×10^{-75} Pa m^3 mol^{-1} (25°C)
P Ka	neutral	4.15
Elimination half-life	25–65 h	2 h
Excretion	72% of oral dosage excreted in urine, 28% in faeces	Biliary excretion: 65% of oral dosage excreted in urine
Metabolites in urine (%of oral dosage)	CBZ, CBZ-epoxide, CBZ-diol, CBZ-acridan, 2-OH-CBZ, 3-OH-CBZ	DFC, 5-OH-DFC, 4´-OH-DFC, 3´-OH-DFC, 4´-5-diOH-DFC, 4´-OH-5-Cl-DFC, 3´-OH-4´-CH_3O-DFC
Dosage	Maintenance usually 800–1200 mg daily	75–150 mg daily
Other Information	Autoinduction, i.e., long term applications increase its metabolism	Dermal applications available

Many anthropogenic origin compounds and WWTP effluents are the significant points of discharges for the presence of endocrine disrupting compounds and residuals of pharmaceuticals in the rivers, streams and surface waters. Hence it is of great interest for eliminating these substances within the WWTP. The aeration tank and the final clarifier form one process unit in the conventional activated sludge plants (CASP) and

the separation of treated sewage and sludge is done in the clarifier through sedimentation. So the capacity of sedimentation is important selection criterion. [41]

In a review of Miege [46], about fate of pharmaceuticals, database was created for the assessment of the occurrence and removal efficiency of pharmaceuticals and personal care products with respect to their quantities. The database has allowed for the identification of the most investigated PPCP in WWTPs and the most persistent ones for obtaining reliable and quantitative values on their concentrations and also to obtain the frequency of the detection and removal efficiency in WWTPs. In the review they have allowed to indentify more than one hundred pharmaceuticals and PPCPs from different prescription classes measured in WWTPs of different countries like Brazil, North America, and European countries. This review contains analgesics and anti-inflammatory drugs, antibiotics and bacteriostatics, anti-epileptics, beta blockers, blood lipid regulators, contrast media, cytostatics, hormones (including oral contraceptives), antidepressants and anxiolitics, musk fragrances, disinfectants and antiseptics. [46]

Table 2.14. The pharmaceuticals and personal care products the most investigated in wastewater treatment plants. [46]

Therapeutic class	Molecules	Frequency (%)a
Hormone	Estrone, 17β-estradiol, 17α-ethinylestradiol, Estriol, 17α-estradiol, Testosterone, Progesterone	30
Analgesic-anti-inflammatory	Ibuprofen, Diclofenac, Naproxen, Ketoprofen,Mefenamic acid	20
Antibiotic	Sulfamethoxazole, Trimethoprim, Ciprofloxacin, Roxithromycin, Norfloxacin, Clarithromycin, Erythromycin	8.7
Lipid regulator	Bezafibrate, Gemfibrozil	4.4
Anti-epileptic	Carbamazepine	4.0
Metabolite	Clofibric acid, Salicylic acid	3.9
Beta-blocker	Metoprolol, Propranolol, Atenolol	2.8
Personal care product	Galaxolide, Tonalide	2.7
Contrast product	Iopromide	1.1
Disinfectant	Triclosan	0.8
Vasodialator	Pentoxifyllin	0.7
Antidepressant	Diazepam	0.6

Citation frequency for paracetamol and 0.3% for aspirin, bisoprolol and sotalol was only 0.6%.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) which is used to reduce inflammation and for relieving pain. It works as an analgesic in cases of arthritis

or acute injury. It is also used to reduce the pain during menstrual pain, dysmenorrhoea. Diclofenac is eliminated in a short period; its elimination half life is about 2 hours [40].

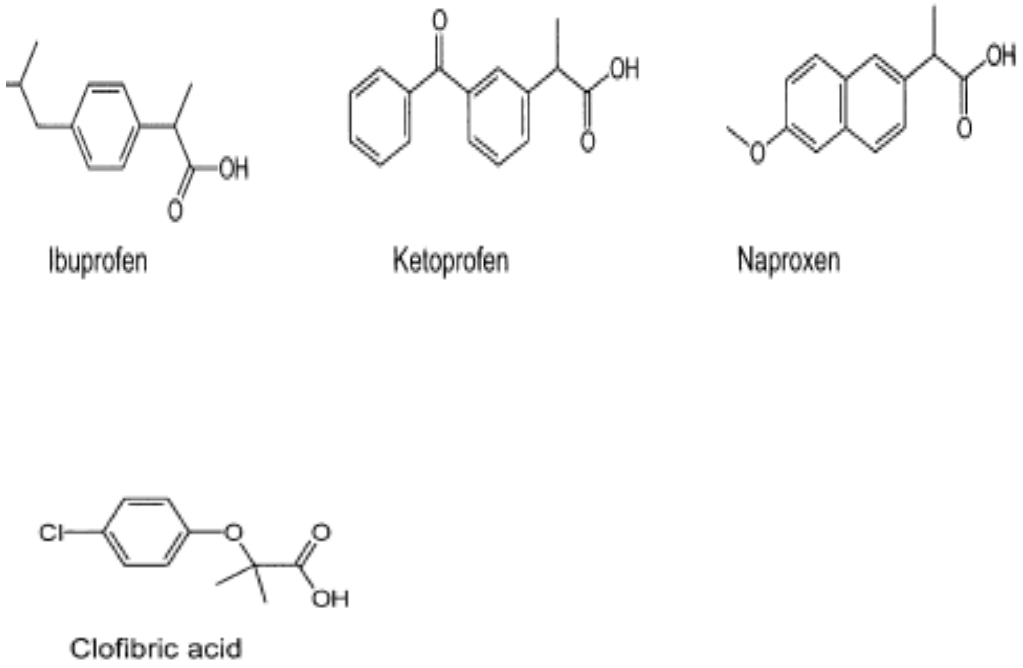


Figure 2.13. Chemical structures of Ibuprofen, Ketoprofen, Naproxen and Clofibric acid. [43]

In a study by Tixier [43] about occurrence and fate of Carbamazepine, Clofibric acid, Diclofenac, Ibuprofen, Ketoprofen and Naproxen in surface waters, continuous concentration measurements were carried out in the effluents of three WWTPs, in two rivers, and in lake over a time period of three months. It was investigated that concentration time courses of the drugs in the effluents of the WWTPs, ranging from the limit of detection (1.5-10 ng/L, depending on the compound) to more than 3 μg/L. All compounds had the pronounced fluctuations in their concentrations. The highest concentrations were found for Naproxen and Ibuprofen in the effluent of WWTP. Clofibric acid and Ketoprofen presented in the lowest concentrations did not exceed 60-180 ng/L. [43]

Diclofenac, a non-steroidal anti-inflammatory drug most commonly detected pharmaceuticals in sewage treatment plant (STP) effluents. Biologically produced manganese oxides (BioMnOx) were investigated to remove Diclofenac. [44]

Toxic Substances Hydrology Program of the U.S. Geological Survey (USGS) shows that many chemicals are found in residential, industrial, and agricultural

wastewaters. Generally they occur in mixtures at low concentrations downstream mainly in the highly populated and animal production areas. Human and veterinary drugs (including antibiotics), natural and synthetic hormones, detergent metabolites, plasticizers, insecticides, and fire retardants are the main chemicals. More than one chemicals were detected in 80 percent of the streams sampled, and 82 of the 95 chemicals were detected at least once. Generally these chemicals were found at very low concentrations (in most cases, less than 1 part per billion chemical mixtures were common; 75 percent of the streams had more than one, 50 percent had 7 or more, and 34 percent had 10 or more . [45]

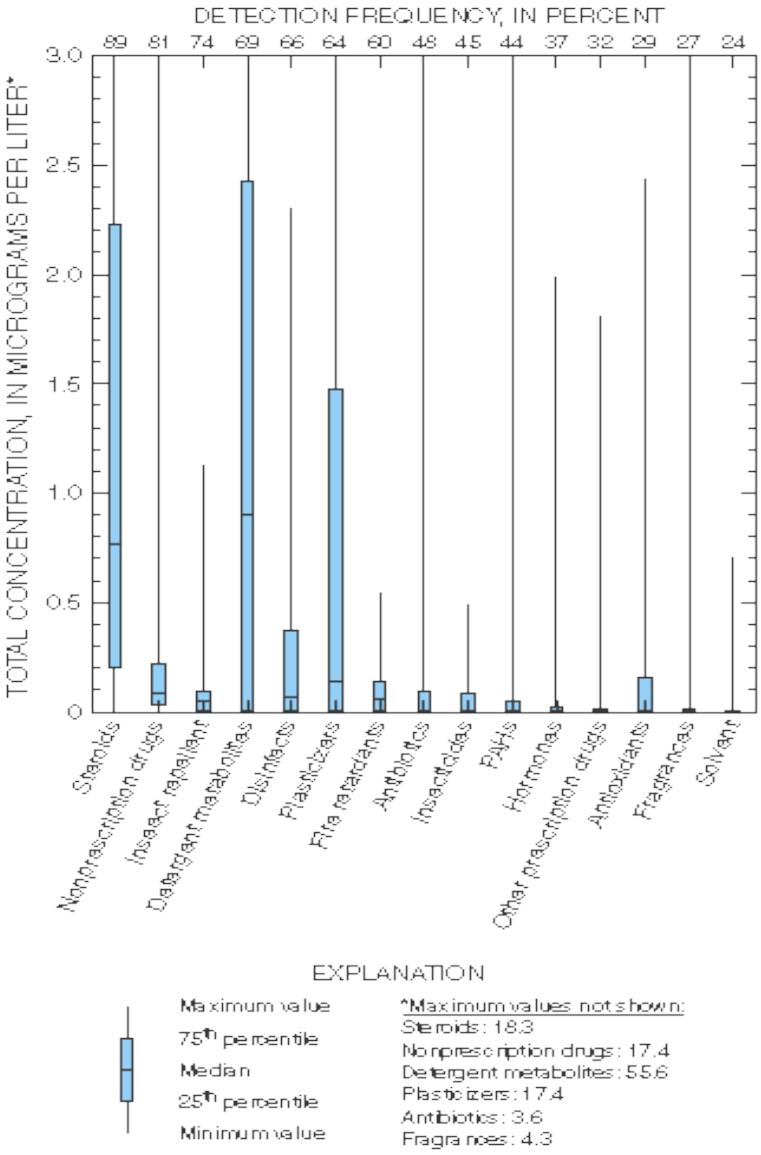


Figure: 2.14. Total concentrations of the chemicals in microgram per liter [45]

Steroids, nonprescription drugs, and an insect repellent were the three chemical groups most commonly detected in susceptible streams. Detergent metabolites, steroids, and plasticizers generally were found at the highest concentrations. [45]

2.6. The toxicity of drugs to the biological purification system

Operation of any biological system is an adequate supply of oxygen is an important factor. Like humans cells also need oxygen to breathe not only the organic material as food. Biological degradation of the waste is not adequate with the less supply of oxygen. There are two types of biological wastewater treatment that are Mechanical method for creating contact between wastewater, cells and oxygen and another is without mechanical means that doesn't include cells and oxygen. [47]

There is a significant transformation of chemicals that occurs in nutrient-removing wastewater treatment plants. The same performance are observed in configurations of the reactor as wide as conventional activated sludge, membrane-bioreactors and suspended-biofilm reactors even though the average hydraulic retention time changes by factors of more than 10. [48]

Activated sludge processes using WWTPs uses microorganisms for mineralizing the pollutants to water and carbondioxide or degrade them to the certain acceptable forms. Pollutants from the water can be removed by air stripping or by sorption onto sludge that is continuously discharged. So the removal of pharmaceuticals residues in activated sludge process consists of four mechanisms: Biotransformation, air stripping, sorption and photo transformation. [40]

Diclofenac has a poor biodegradation rate. The sorption behavior of Diclofenac onto sludge is same like Carbamazepine. Removal efficiency of Diclofenac could be upto 80% and the carbamazepine below 10%. This is because the Carbamazepine is extraordinarily persistent to biodegradation at low concentrations and the biodegradation of Diclofenac may be possible under some conditions. [40]

Ibuprofen doesn't contain chlorine and double aromatic rings also absent in its structure. This makes it easier for the degradation. Removal of clofibric acid is poor as it contains chlorine in its structure. It is identified as a refractory contaminant in many investigations of municipal sewage influents and effluents. Membrane bioreactors are the efficient process for removal of Clofibric acid which would increase the feasibility of the technology. MBR is an advanced technology for the wastewater treatment process. [50]

Table 2.15 below shows the toxicity data of Carbamazepine and Diclofenac in the literatures. The table shows the toxicity data for bacteria, algae, microcrustaceans and fish. Acute toxicity with EC50 concentrations, chronic toxicity with no observed effect concentration (NOEC) and predicted no-effect concentrations (PNEC) have been mentioned. Regarding predicted no-effect concentrations (PNEC), Carbamazepine has shown more hazardous effect showing the value of 0.42 $\mu g\ L^{-1}$. [40]

Table 2.15. Toxicity data of Carbamazepine and Diclofenac in the literatures. [40]

	Acute toxicity EC50	Chronic toxicity NOEC	PNEC	References
Carbamazepine	>13.8–81 mg L^{-1}	25–100 mg L^{-1}	0.42 µg L^{-1}	Ferrari et al.(2003)
	1–10 mg L^{-1}		6.359 µg L^{-1}	Jones et al.(2002)
	4.5–383.5 mg L^{-1}	1–10 mg L^{-1}	116 µg L^{-1}	Jos et al. (2003)
Diclofenac	75.1–502.6 mg L^{-1}		138.74 µg L^{-1}	Lavilleet al.(2004)
	11.5–22.7 mg L^{-1}			Ferrari et al.(2003)
	1–10 mg L^{-1}			Jones et al.(2002)
	3.3–142.2 mg L^{-1}			Lavilleet al.(2004)
	90 ± 20 µg L^{-1}on zebra fish embryos.			Dietrich and Prietz (1999)
	68 mg L^{-1}	45 mg L^{-1}		Cleuvers (2004)
		1mg L^{-1}on Daphnia magna and 1 µg L^{-1}on histopathological lesions		Schwaiger et al. (2004)

EC50: concentrations that cause 50% of effect.
NOEC: no observed effect concentration.
PNEC: predicted no-effect concentrations.

The removal ability of each treatment process was evaluated based on the removal rate, calculated from the following expression

$R\ (\%) = (Cin - Cout) / Cin \times 100$

Where, *Cin* and *Cout* are the concentration of the selected compounds in the raw wastewater and the effluent of each process, respectively. [49]

Table 2.16. Influent and effluent concentrations and removal efficiency in sewage treatment plants (different equipment, different countries, sampling in different seasons. [67]

Compound	Influent concentration (μg/L)	Effluent concentration (μg/L)	Maximal removal (%)	Reference
Analgesics and antiinflammatory drugs				
Acetylsalicylic acid	3.2	0.6	81	Ternes et al. (1999)
Salicylic acid	57	0.05	99	Metcalfe et al. (2003a)[a]
	330	3.6		Carballa et al. (2004)
Dextropropoxyphene	0.03	0.06	0	Roberts and Thomas (2005)[a]
Diclofenac	3.0	2.5	17	Heberer (2002)
	n.r.	n.r.	69	Ternes (1998)[b]
	0.33–0.49	n.r.	75 (10–75)	Andreozzi et al. (2003a)[c]
	[5]	[1.5]	53–74	Strenn et al. (2004)[a]
	1.3	n.r.		Metcalfe et al. (2003a)[a]
	0.47–1.9	0.31–0.93		Buser et al. (1998b)
	2.8	1.9	23 ± 30	Quintana et al. (2005)[b]
	0.4–1.9	0.4–1.9	0	Tauxe-Wuersch et al. (2005)[c]
	0.35 ± 0.1	0.17–0.35	9–60	Lindqvist et al. (2005)[c]
	1.0	0.29	71	Roberts and Thomas (2005)[a]
Ibuprofen	3		96	Buser et al. (1999)
	38.7	4	>90	Metcalfe et al. (2003a)[a]
	9.5–14.7	0.01–0.02	99	Thomas and Foster (2004)
	[0.54]	[0.08–0.28]	22–75 99 (52–99)	Andreozzi et al. (2003a)[c]
	[1.5]	[0.01]	12–86	Strenn et al. (2004)[a]
	2.6–5.7	0.9–2.1	60–70	Carballa et al. (2004)[a]
	5.7	0.18	97 ± 4	Quintana et al. (2005)[b]
	28.0	3.0	98	Roberts and Thomas (2005)[a]
	2–3	0.6–0.8	53–79	Tauxe-Wuersch et al. (2005)[c]
	13.1 ± 4	0–3.8	78–100	Lindqvist et al. (2005)[c]
Ketoprofen	0.41–0.52	0.008–0.023	98	Thomas and Foster (2004)
	[0.55]	[0.18–0.3]	48–69	Stumpf et al. (1999)[b]
	5.7	n.r.		Metcalfe et al. (2003a)[a]
	0.47	0.18	62 ± 21	Quintana et al. (2005)[b]
	0.25–0.43	0.15–0.24	8–53	Tauxe-Wuersch et al. (2005)[c]
	2.0 ± 0.6	0–1.25	51–100	Lindqvist et al. (2005)[c]
Mefenamic acid	1.6–3.2	0.8–2.3	2–50	Tauxe-Wuersch et al. (2005)[c]
Naproxen			66	Ternes (1998)[b]
	40.7	12.5	40–100	Metcalfe et al. (2003a)
	10.3–12.8	n.d.-0.023	100	Thomas and Foster (2004)
	[0.6]	[0.1–0.54]	15–78	Stumpf et al. (1999)[b]
			93 (42–93)	Andreozzi et al. (2003a)[c]
	1.8–4.6	0.8–2.6	40–55	Carballa et al. (2004)[a]
	0.95	0.27	71 ± 18	Quintana et al. (2005)[b]
	4.9 ± 1.7	0.15–1.9	55–98	Lindqvist et al. (2005)[c]
Paracetamol	6.9	0	100	Roberts and Thomas (2005)[a]
β-Blocker				
Metoprolol	n.r.	n.r.	83	Ternes (1998)[b]
	n.r.	n.r.	10 (0–10)	Andreozzi et al. (2003a)[c]

Propranolol	n.r.	n.r.	96	Ternes (1998)[b]
	70	304	0	Roberts and Thomas (2005)[a]
Atenolol	n.r.	n.r.	<10 (0–10)	Andreozzi et al. (2003a)[c]
Blood lipid lowering agents				
Bezafibrate	[1.18]	[0.6–0.84]	27–50	Stumpf et al. (1999)[b]
	n.r.	n.r.	83	Ternes (1998)[b]
	[5]	[0.01]	10–97	Strenn et al. (2004)[a]
	0.6	0.2		Metcalfe et al. (2003a)[a]
	2.6	0.24	91 ± 4	Quintana et al. (2005)[b]
	0.42 ± 0.3	0–0.85	15–100	Lindqvist et al. (2005)[c]
Gemfibrozil	n.r.	n.r.	69	Ternes (1998)[b]
	[0.3]	[0.18–0.28]	16–46	Stumpf et al. (1999)[b]
	n.r.	n.r.	75 (10–75)	Andreozzi et al. (2003a)[c]
	0.7	1.3	n.r.	Metcalfe et al. (2003a)[a]
Fenofibric acid	[0.44]	[0.22–0.4]	6–45	Stumpf et al. (1999)[b]
	n.r.	n.r.	64	Ternes (1998)[b]
Clofibric acid	n.r.	n.r.	6–50	Stumpf et al. (1996)
	[1]	[0.68–0.88]	15–34	Stumpf et al. (1999)[b]
	n.r.	n.r.	51	Ternes (1998)[b]
	0.15–0.25	0.15–0.25	0	Tauxe-Wuersch et al. (2005)[c]
	0.34	0	91	Roberts and Thomas (2005)[a]
Neuroactive compounds				
Carbamazepine	n.r.	n.r.	7–8	Ternes (1998)[b]
	0.7	0.7	<50	Metcalfe et al. (2003a)[a]
	n.r.	n.r.	8	Heberer (2002)
	[1.5]	n.r.	4	Clara et al. (2004)[a]
	n.r.	[1.5]	53 (0–53)	Andreozzi et al. (2003a)[c]
Diazepam	0.59–1.18	0.1–0.66	93	Van Der Hoeven (2004)
Various				
Ethinylestradiol	0.003	0.0004	85	Baronti et al. (2000)
Clotrimazole	0.031	0.14	55	Roberts and Thomas (2005)[a]
Ifosfamide	0.007–0.029	0.010–0.043	0	Kümmerer et al. (1997)[a]
Tamoxifen	0.15	0.20	0	Roberts and Thomas (2005)[a]
X-ray contrast media	0.18–7.5	0.14–8.1	0	Ternes and Hirsch (2000)[b]

Data estimated from graphical data are in square brackets. n.r.: not reported.
a Median concentrations or percent.
b Average concentrations or percent.
c Maximal concentrations or percent.

In Table 2.16, Influent and effluent concentrations and removal efficiency in sewage treatment plants (different equipment, different countries, sampling in different seasons) of different pharmaceuticals are shown. The average elimination for particular pharmaceuticals ranges from only 7-8 % for Carbamazepine upto 81% for Acetyl salicylic acid, 96% for Propranolol and 99% for Salicylic acid. Lowest average removal rates were found for Diclofenac as 26%, for Bezafibrate 51%. This varies significantly between STPs. Naproxen was found having high removal rate of 81%. This table shows that removal rates are not same even for the same pharmaceutical between different plants. 94-100% of Ibuprofen, Naproxen, Ketoprofen and Diclofenac was found in three STPs in the U.S.A. Mainly in the secondary treatment step, efficient removal takes place about 51-99% but in primary treatment only 0-44% were removed. [67]

Gros [51] have studied removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Pharmaceuticals occur in WWTP effluents as they do not have tendency to adsorb onto activated sludge or because the microbial degradation may not be rapid to be completed in the hydraulic retention time of the plants. The range of removal rates (%RE) for the most representative compounds of each therapeutic group, in the whole set of WWTP under investigation is listen in Table 2.17. [51]

Table 2.17. The range of removal rates (%RE) for the most representative compounds of each therapeutic group, in the whole set of WWTP under investigation. [51]

Compounds	**Range of %RE**	**Average %RE (± RSD)**
Sulfadiazine	[43–98]	69 (± 32)
Sulfamethoxazole	[30–92]	74 (± 22)
Norfloxacin	[30–98]	57 (± 54)
Ofloxacin	[20–99]	40 (± 64)
Ciprofloxacin	[37–99]	66 (± 35)
Tetracycline	[40–89]	71 (± 33)
Enalapril	[83–99]	96 (± 11)
Salbutamol	[20–99]	60 (± 44)
Famotidine	[30–99]	50 (± 59)
Ranitidine	[50–98]	66 (± 39)
Cimetidine	[30–99]	50 (± 64)
Glibenclamide	[22–75]	46 (± 39)
Nadolol	[25–99]	60 (± 51)
Atenolol	[20–97]	59 (± 50)
Bezafibrate	[23–99]	69 (± 39)
Gemfibrozil	[30–99]	67 (± 48)
Atorvastatin	[40–80]	58 (± 44)
Propyphenazone	[30–87]	44 (± 68)
Ketoprofen	[40–100]	69 (± 40)
Naproxen	[60–100]	86 (± 13)
Ibuprofen	[65–100]	91 (± 13)
Diclofenac	[30–100]	58 (± 53)
Acetaminophen	[96–100]	99 (± 1)
Salicylic acid	[82–99]	96 (± 8)
Furosemide	[20–96]	50 (± 59)

There was an increase in concentration of the drugs along the passage through the WWTPs. The investigation showed that Macrolide antibiotics, Carbamazepine, Benzodiazepines and Serotonin reuptake inhibitors showed poor or no elimination in all WWTP. Diclofenac, as an exception, the removal rate was varied from no elimination upto 100%. The increase of Carbamazepine concentration in wastewater effluent occurs due to conversion of Carbamazepine glucoronides and other conjugated metabolites to the parent compound by enzymatic processes which happen in the treatment plant. When Lipid regulators, Fluoroquinolones, Tetracycline antibiotics are detected, and also

cholesterol lowering statin drugs, histamine H1 and H2 receptor antagonists, β-blockers, β-agonists and the anti-diabetic glibenclamide were partially degraded which showed the removal efficiencies between 40 and 60–70%. [51]

In one of the study by Quinn [52], the toxicity data of the pharmaceuticals were interpreted by using EU directive 93/67/EEC that classifies substances according to the measured effective concentrations (EC50 value). When this scheme was applied to the data that was obtained in their study Gemfibrozil, Ibuprofen and Naproxen were classified as toxic which have an EC50 between 1 and 10 mg/L. Carbamazepine, Bezafibrate, Sulfapyridine, Oxytetracycline and Novobiocin were all classified as harmful (EC50 between 10 and 100 mg/L) and Sulfamethoxazole, Trimethoprim and Caffeine were considered non-toxic (EC50N100 mg/L) as show in the table 2.18. These results are similar to other studies that included Ibuprofen, Naproxen, Gemfibrozil and Carbamazepine as high risk pollutants in WWTP effluents. [52]

Table 2.18. Level of toxicity for each of the 11 pharmaceuticals under investigation, based on the chronic EC50 results from Hydra attnuata in the current study using classification from EU directive 93/67/EEC. [52]

Pharmaceutical	Extremely toxic	Very Toxic	Toxic	Harmful	Non-toxic
	EC_{50} < 0.1 mg/L	EC_{50} < 0.1–1 mg/L	EC_{50} 1 – 10 mg/L	EC_{50} 10 – 100 mg/L	EC_{50} > 100 mg/L
Gemfibrozil					
Ibuprofen					
Naproxen					
Carbamazepine					
Bezafibrate					
Sulfapyridine					
Oxytetracycline					
Novobiocin					
Sulfamethoxazole					
Trimethoprim					
Caffeine					

Table 2.19. Acute (LC50) and Chronic (EC50, LOEC, NOEC) responses (mg/L) with 95% confidence interval (CI) based on morphology for Hydra attenuate exposed for 96h to the 11 pharmaceuticals under investigation. [52]

Pharmaceutical	**96 h LC50**	**95% CI**	**96 h EC50**	**95% CI**	**LOEC**	**NOEC**	**TT**
Carbamazepine	29.4	(32.83–26.32	15.52	(29.02–8.3)	5	1	2.24
Bezafibrate	70.71	(70.71–70.71	25.85	NC	1	0.1	0.32
Sulfapyridine	> 100	NC	21.61	(34.23–13.64)	5	1	2.24
Oxytetracycline	> 100	NC	40.13	(46.95–34.3)	100	50	70.71
Novobiocin	> 100	NC	NC	NC	100	50	70.71
Sulfamethoxazole	> 100	NC	NC	NC	10	5	7.07
Trimethoprim	> 100	NC	NC	NC	> 100	> 100	NC
Caffeine	> 100	NC	NC	NC	> 100	> 100	NC

NC= not calculable, results didn't fit the appropriate test. Toxicity threshold

TT= $(NOEC \times LOEC)^{1/2}$

Acute toxicity was based on the effect on morphology where the LC50 and EC50 values with their 95% confidence limits, are shown in above Table 2.19.

2.7. Different methods of drugs removal from wastewater treatment plant

DuPont has developed the PACT^{TM} process in the early 1970s for the removal of color from industrial wastewater. PAC (Powdered Activated Carbon) was added to an activated sludge reactor for removing compounds which were not degraded by the microorganisms and for providing better treatment which can only be obtained with tertiary treatment process. The study with PACT^{TM} has been used to determine whether the IC_{50} value for each specific inhibitory compound was greater for PACT^{TM} sludge or activated sludge and to determine whether the carbon concentration and sludge age combination impacted the IC_{50} value. PACT^{TM} has been claimed to have the different benefits over conventional activated sludge systems. [53]

- Improved process stability during shock loads by adsorption of soluble organic compounds
- Improved COD removal by adsorption of non-biodegradable organic compounds
- Improved color removal
- Improved sludge settling, thickening and dewatering characteristics
- Improved hydraulic capacity which is may be due to increased removal rates or operation with higher mixed liquor biomass levels

- Improved nitrification by either adsorbing inhibitors or providing a surface for the attachment of nitrifiers and
- Improved removal of EPA priority pollutants. [53]

Ozonation is one of the advanced techniques for waste water treatment and title of many recent Studies. Formation of OH radicals due to ozone decay in the water is the main mode of action in the Ozonation process, but there is also ozone molecules present for chemical attack. So the oxidation capacity will be increased. No oxidant residues remain as an advantage.

Ozonation has a good performance in removal of pharmaceuticals. 50% of the pharmaceuticals were removed during ozonation of conventional activated sludge (CAS) and membrane bioreactor (MBR) effluents with different ozone doses was shown in a study done in Germany. Sand Filtration (SF), Ozonation, Microfiltration/reverse osmosis (MF/RO) and Ultrafiltration are the other methods to eliminate the target compounds in the wastewater treatment plants. In a research by Sui [54], these methods were employed in two WWTPs for eliminating target compounds which proved very effective with the main contributions in removing micropollutants in wastewater treatment. [54]

Table 2.20. Removal efficiencies (%) of target pharmaceuticals and consumer products by advanced treatment process in studied WWTPs. [54]

COMPOUND	WWTP A		WWTP B	WWTP D
	UF	OZONE	SF	MF/RO
DEET	0–50	50–80	0–50	>90
CF	<0	50–80	<0	50–80
TP	0–50	>90	80–90	>90
BF	0–50	0–50	0–50	>90
CA	<0	50–80	<0	80–90
GF	0–50	80–90	50–80	>90
DF	0–50	>90	<0	>90
IM	0–50	>90	<0	>90
MA	0–50	80–90	<0	0–50
MTP	0–50	80–90	<0	>90
CBZ	<0	>90	0–50	>90
SP	0–50	>90	0–50	>90

Table 2.20 shows the removal efficiencies (%) of target pharmaceuticals and consumer products by advanced treatment process in studied WWTPs. Higher HRT (Hydraulic Retention Time) (›12h) and SRT (Solid Retention Time) (›10d) can contribute to an increased removal rate of pharmaceuticals. In the secondary treatment, the average removal rate for different compounds ranged from -12% to 100%. Caffeine, Benzafibrate, Trimethoprim and DEET (N, N-Diethyl-meta-toluamide) were removed effectively with the average efficiency of 100 ±0%, 88±12%, 76±24% and 69 ±21% respectively. Caffeine was proved to be readily biodegradable. 28-53% of Diclofeanc was removed by a secondary treatment in the WWTPs that was between 26% in Finland and 69% in Germany. [54]

O_3 and O_3/ H_2 O_2 processes are better for the treatment of pharmaceutical wastewater containing antibiotics. Investigations showed that Ozonation process is able of achieving high levels of COD and aromaticity removals at about their natural pH values. Mostly biodegradable fraction of wastewater can be increased by Ozonation that leads to the formation of low molecular weight oxygenated byproducts which are more amenable to biodegradation. Ozonation could be successfully used as a pretreatment step for improving the biodegradability of wastewater containing antibiotics. [55]

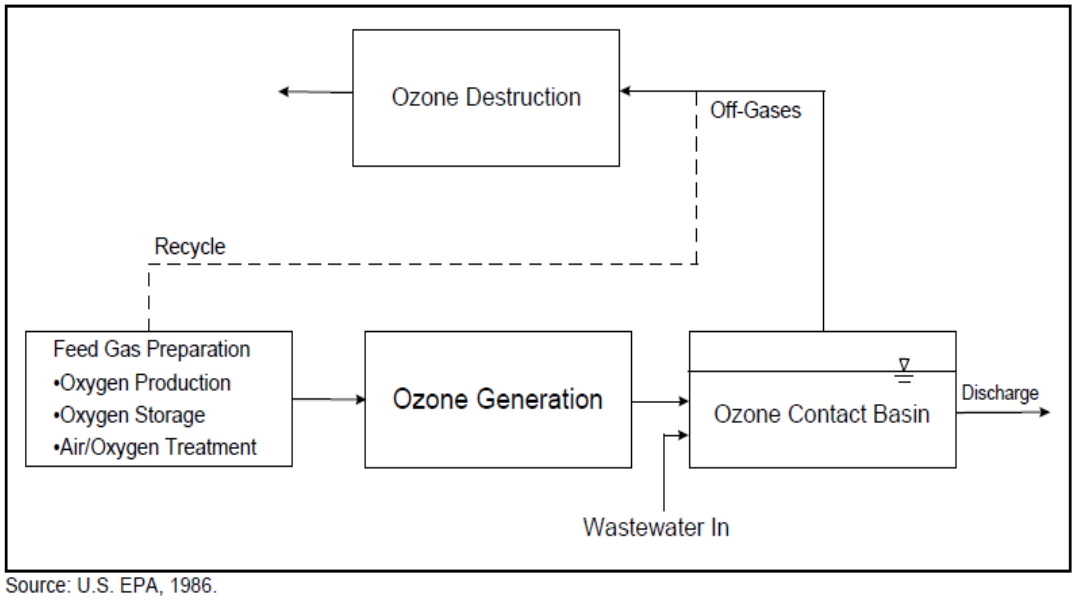


Figure 2.15 Ozone process schematic diagram. [57]

Figure 2.15 describes the working mechanism of the Ozone process. There are different steps shown in the schematic diagram which consist of ozone generation and wastewater in and other intermediate steps. Ozone is effective to destroy viruses and bacteria and it is more effective than chlorine. Ozonation process takes a very short contact time, approximately 10 -30 minutes. Ozonation process doesn't leave harmful residuals as ozone decomposes very fast. Ozonation doesn't let the microorganisms to regrow. [57]

Coagulation-flocculation and floatation is the another method to remove drugs from wastewater treatment. It is a physical means for enhancing separation in which metal salts like iron (III), chloride, or aluminum phosphate are added to water. This allows the precipitation of suspended solids and colloids. Lipophilic trace pollutants are adsorbed on colloids. This is the optional technique to remove the nonpolar pharmaceuticals from the wastewater. Floatation is used for separating fine solid particles from the aqueous phase by adhering them to the surface of upstreaming bubbles of air. This is also used to remove non-polar pharmaceuticals. Lipophilic compounds like Diclofeanc can be removed upto 70% during coagulation-flocculation method because of their significant sorption affinity. [58]

Coagulation and flocculation process form removal of pharmaceuticals depends on a compound's propensity to sorb to surfaces. As a result, hydrophobic compounds with high octanol-water partition coefficients (log Kow) can potentially be removed by coagulation/flocculation. But many pharmaceuticals are polar which have a smaller tendency to sorb to surfaces. For example, the log Kow values for Sulfamethoxazole and sulfamethazine are 0.89 and 0.28 respectively. So, their removal by coagulation is not significant. [60]

In comparison with ozone-based Advance Oxidation Processes (AOPs), there are additional advantages of UV/ H_2 O_2 processes. They are: (1) relatively easy H_2 O_2 storage and high thermal stability, (2) infinite H_2 O_2 solubility with water and no gas-liquid mass transfer limitations,(3) reactions with organic compounds form peroxyl radicals which are also reactive that leads to succeeding oxidation reactions and (4) rapid installation and simple operation which is good for small water treatment plants . [60]

Fenton System

Researchers are interested in separating the source of the refractory or toxic effluent and treat it by advanced oxidation processes (AOPs) by using homogeneous or heterogeneous catalyst. Fenton system Fe^{n+}/H_2O_2 is one of the most interesting oxidative techniques for the abatement of refractory and toxic organic pollutants in water and wastewater .This technique has the high removal efficiency which includes the formation of strong hydroxyl radical (HO) and oxidation of Fe^{2+} to Fe^{3+}. Fe^{2+} and Fe^{3+} ions are both coagulants, therefore the Fenton process has dual function, oxidation and coagulation in the treatment processes. Iron is a easily available and non-toxic element. Also, hydrogen peroxide is environmental friendly. So this process has good efficiency in treatment processes for the removal of the refractory organic compounds from pharmaceutical industrial wastewater before being discharged into sewerage system. [62]

Anaerobic digestion

Anaerobic digestion is another method which is particularly used for sewage treatment. It is contaminated with pharmaceuticals due to the municipal sewage origin. During the sludge cycling, pharmaceuticals are adsorbed to the sludge or dissolve in the high water content (>90%) can accumulate in the STP. This is the additional sludge digestion process where organic matter is degraded anaerobically under mesophilic (37.5°C) or thermophilic (55.5°C) conditions by a microorganism community. Pharmaceuticals being adsorbed to the sludge or dissolved in the high water content (>90%) can accumulate in the STP during the sludge cycling or in the environment after disposal. Methanogenesis is the main metabolism process in anaerobic sludge digestion, which is not affected by Carbamazepine and Sulfamethoxazole in concentrations up to 400 mg/l. But Diclofenac can inhibit this process at high concentrations. [61]

Pharmaceuticals investigated in the study of Carballa [61], were affected by the anaerobic digestion where Sulfamethoxazole (99%), Diclofenac (69%), Ibuprofen (41%), Iopromide (25%) were the average removal detected. They assumed that an increase in removal for some compounds like Diclofenac whose removal was increased to 80 % by extending the contact time. This was because of an increase in biodiversity of the degraders. The temperature of the process had a minor effect to pharmaceutical removal instead. [61]

2.8. Fate and Effects of Pharmaceuticals in the environment

The main effect of the occurrence and fate of pharmaceutically active compounds (PhACs) is in the aquatic environment. It is one of the emerging issues in environmental chemistry. In the countries like Austria, Brazil, Canada, Croatia, England, Germany, Greece, Italy, Spain, Switzerland, The Netherlands, and the U.S., more than 80 compounds, pharmaceuticals and several drug metabolites, have been detected in the aquatic environment. [63]

In sewage influent and effluent samples and in different surface waters situated downstream from municipal sewage treatment plants, the pharmaceuticals have been detected upto gram per liter level. Insufficient treatment of the wastewater results in the contamination of drugs in the receiving water bodies. Polar PhACs such as Clofibric acid, Carbamazepine, Primidone or Iodinated contrast agents can leach through the subsoil. They are detected in several groundwater samples in Germany, under recharge conditions. [63]

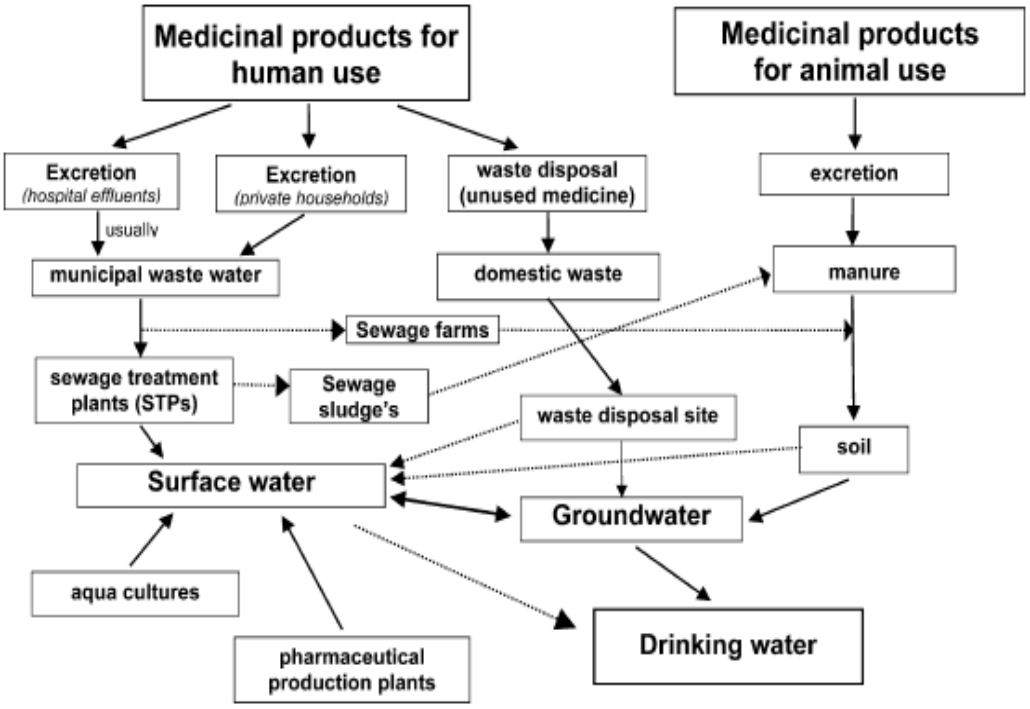


Figure 2.16. Scheme showing possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. [63]

Figure 2.16 shows the schematic diagram for the possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. The possible sources are the medicines used for the human use and the animal use. Medicinal product for animal use occurs through excretion and waste disposal and also the medicines for animal use come in contact with soil through excretion. These medicinal products finally mix with the surface water and ground water. These are then received into drinking water, aqua cultures and pharmaceutical production plants. [63]

'Metabolite' word is used for compounds that are resulted from the structural change of pharmaceuticals within the human body and not differentiating biochemical processes performed by human enzymes because of the bacterial activity in the gut and the ones that are present in the skin or non-biotic processes like hydrolysis in the stomach. So the metabolites may be formed by biological or non- biological processes.

The chemical structure of the active molecules can be changed with the metabolism process, by biotransformation, biodegradation, and non-biotic transformation such as photo transformation and hydrolysis. Such a structural change brings a change in their physico-chemical and pharmaceutical properties. This phenomenon helps in the elimination of pharmaceuticals easily. [64]

Generally, effluent from drug manufactures contains extremely high levels of pharmaceuticals. Drugs are commonly detected in effluents at levels from below 1 ng/L

up to a few µg/L. Reduced overall performance of the treatment plant is due to the toxicity of the pharmaceuticals to the microorganism in the plant. Mixture of residual fluoroquinolones may severely affect the microbial flora downstream from the plant. So there are many reasons of treatment process other than normal biological treatment for the removal of high levels of antibiotic residues from wastewater. [65]

Occurrence of pharmaceuticals in the environment causes ground water pollution. The findings and the distribution of organic compounds originating from waste from the pharmaceutical industry in the down gradient of a landfill. Contamination of tap water by Clofibric acid is a common example. Another is the river water pollution, sediment pollution. Different findings have shown the presence of antibiotics in sediment cores from medication in fish farms. Ocean pollution and the soil pollution are the other effects of pharmaceuticals. Soil pollution can be caused by the use of manure to agricultural soils, multiple drug resistance developed in livestock micro flora. [65]

3. Materials and Methods

The laboratory work can be classified as experimental set up, OUR and MLSS procedure, screening of Active pharmaceuticals and selection of inhibitory compounds, calculation of OUR, calculation of MLSS and MLVSS and calculation of EC_{50} concentration.

3.1. Sample collection

Different pharmaceuticals were obtained from the pharmaceutical company called Universal Corporation Limited, Nairobi, Kenya. It is an investee company of Finland. The company produces off- patent generic drugs, including AIDS, Malaria and Tuberculosis treating drugs. The samples arrived in the laboratory in January 2010. Active ingredients were made into different solutions according to their solubility and standard concentrations. [68]

Different solutions were made and brought in the laboratory for OUR measurements. OUR measurements, MLSS measurements, MLVSS measurements were done for all the samples inorder to check the toxicity of the samples towards activated sludge. Activated sludge was brought from the Tampere Wastewater treatment plant.

3.2. Drugs

Drugs were delivered in the laboratory in four batches. Each batch contains certain numbers of pharmaceuticals. The names of the pharmaceuticals and the number batches are mentioned with their serial number and concentration in a table form

Table 3.1. First batch of Chemicals

S.N	Raw Materials	Concentration (g/L)
160	Ai Quinine Sulphate B.P	0,5
61	Ai Diclofenac Diethyl Ammonium	1
37	Ai Cetrimide	1
120	Ai Metformin Hcl	1
19	Ai Ascorbic Acid BP	1
44	Ai Chlorpheniramine Maleate	1
231	Ex Caustic Soda Pearls(Sodium Hydroxide)	1
85	Ai Folic Acid	0,5
33	Ai Caffeine 1 / Caffeine Anhydrous 2	1
10	Ai Ammonium Chloride	1
106	Ai Levamisole HCL	1
63	Ai Diclofenac Sodium	1
228	Ex Camphor	0,5
166	Ai Salicylic Acid	1
65	Ai Diphenhydramine HCL	1
356	Ex Sugar White	1
92	Ai Guaiphenesin B.P	1
55	Ai Cyanocobalamine B.P/ U.S.P	1
82	Ai Ferrous Sulphate	1
20	Ai Aspirin	1
33	Ai Caffeine 1 / Caffeine Anhydrous 2	1
71	Ai Ephedrine HCL B.P	1

Table 3.2. Second batch of Chemicals

S.N	Raw Materials	Concentration (g/L)
137	Ai Paracetamol BP (Acetaminophen)	1
38	Ai Cetrizine (Cetirizine, Zyrtec)	0.1
96	Ai Ibuprofen 20 Microns	0.01
115	Ai Mebevarine B.P	1
12	Ai Amodiaquine HCl	1
159	Ai Quinine Dihydro Chloride	0.1
143	Ai Phenytoin Sodium	0.01
68	Ai Doxycycline Hcl	0.1
9	Ai Amminosidine (Paromomycine)	1
98	Ai Ibuprofen B.P	0.01

Table 3.3. Third batch of Chemicals

S.N	Raw Materials	Concentration (g/L)
165	Ai Salbutamol Sulphate	1
26	Ai Benzoic Acid	1
105	Ai Lamivudine	1
133	Ai Omeprazole Magnesium	0.1
147	Ai Potassium Chloride	1
40	Ai Chloramphenicol B.P	1
21	Ai Atenolol	1
36	Ai Cimetidine	1

Table 3.4. Fourth batch of Chemicals

S.N	Raw Materials	Concentration (g/L)
149	Ai Povidone Iodine B.P (PvP-iodine, Betadine)	1
94	Ai Hydrocotisone Acetate	0.1
185	Ai Thiamine HCL (B1) B.P	1
242	Ex Citric Acid (anhydrous)	1
127	Ai Nicotinamide B.P	1
332	Ex Propyl Paraben Sodium	1
326	Ex Povidone K- 30	1
197	Ai Zidovudine	1
163	Ai Riboflavine B.P	1
186	Ai Thiamine Mononitrate	1

3.3. Experimental set up

Different solutions and apparatus required for carrying out the experiment was set up on the bench in the laboratory. The apparatus that were used in performing the test are as follows:

Glasswares

- Glass pipette
- Erlenmeyer flasks with stoppers (100 mL,200mL,500mL and 1000 mL)
- Volumetric flasks (500 mL and 100 mL)
- Beakers
- Magnetic stirrers
- BOD bottles (500 mL)
- Pasteur pipettes
- Measuring cylinder
- Stop watch
- Container with activated sludge

Those glassware were used in each step while performing the OUR test and MLSS procedure. OUR apparatus was made ready in the working bench. Solutions to be tested were stored in the cold room.

Apparatus and Solutions for carrying out OUR, MLSS and MLVSS procedures are described below.

3.4. OUR and MLSS methods

3.4.1 OUR method

Apparatus needed:

- Test vessel of 293 ml with a cover
- Oxygen meter WTW Multiline P4
- Oxygen probe CellOx325
- Magnetic stirrer
- Overflow bowl
- Laptop with WTW MultiAchat II program
- 1 L volumetric flask
- Measuring cylinder for activated sludge
- 500 mL Erlenmeyer flask.

A nutrient solution was prepared by dissolving the following ingredients into 1000 mL tap water in a volumetric flask. The constituents and the quantities are shown below in Table 3.5

Table 3.5. Nutrient solution

Constituents	Quantity
Peptone	16g
Dextrose $C_6H_{12}O_6$	16g
Urea $OC(NH_2)_2$	3g
Dipotassium hydrogen phosphate K_2HPO_4	2.8g

Preparation of OUR equipment

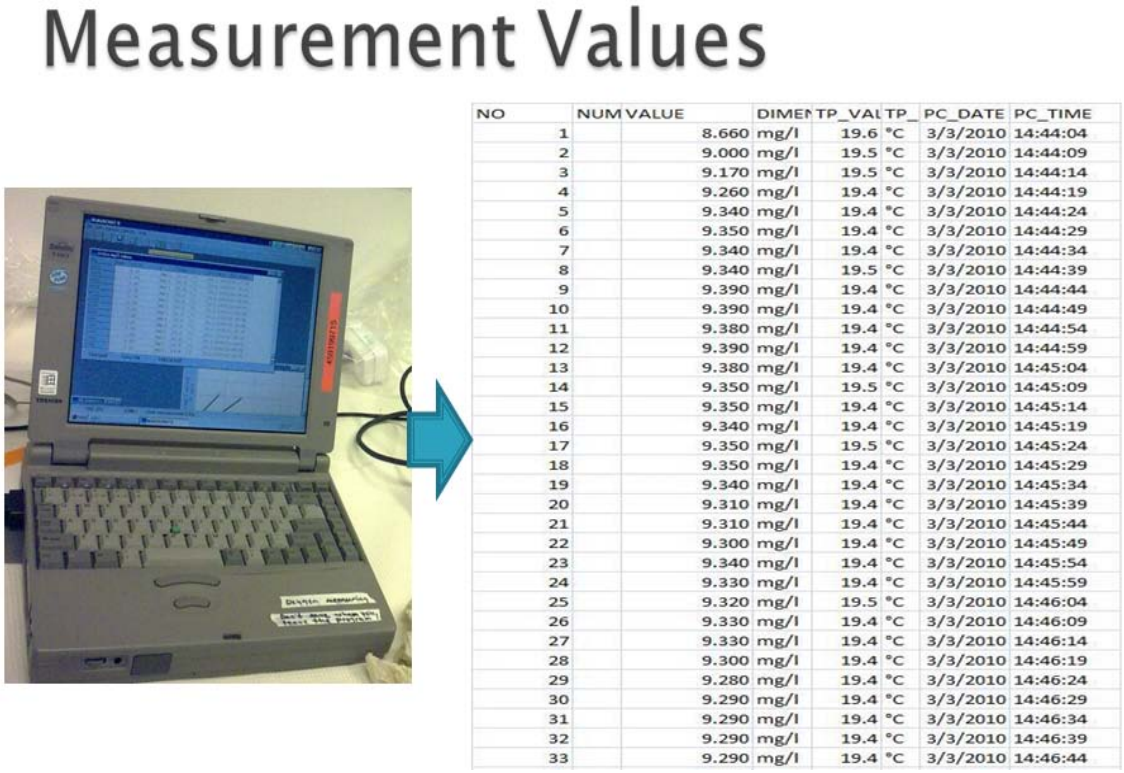
MultiAchatII program was started. At first, Start was selected and then Select- meter-COM and Configuration. Kind: Oxygen was choosed and the Type: Oxi325. Interface was choosed as COM1. File was choosed and clicked save continuously and then file was named (e.g.100216.dbf). Edit was choosed and right mouse button was clicked in the Graphics window. Configure was choosed as Carriage 1Spc/h and timer was clicked and choosed 10 s between measurement.

Oxygen meter was calibrated while the CellOx325 probe was in the white storage sleeve. CAL was pressed and RUN/ENTER was clicked. The AR sign started flashing and O2 was pressed when AR stopped flashing. The device was ready for the measurement of Dissolved Oxygen (DO) level.

Injection volume required for each measurement was calculated for each chemical, blank and sludge with chemical. The stock standards of the active ingredients were made ready as working standard for OUR measurements.

Figure 3.1. The test equipment for OUR determination (a)

Figure 3.1 shows the test equipment for determination of OUR which was used in the laboratory.



NO	NUM	VALUE	DIMEN	TP_VAL	TP_	PC_DATE	PC_TIME
1		8.660	mg/l	19.6	°C	3/3/2010	14:44:04
2		9.000	mg/l	19.5	°C	3/3/2010	14:44:09
3		9.170	mg/l	19.5	°C	3/3/2010	14:44:14
4		9.260	mg/l	19.4	°C	3/3/2010	14:44:19
5		9.340	mg/l	19.4	°C	3/3/2010	14:44:24
6		9.350	mg/l	19.4	°C	3/3/2010	14:44:29
7		9.340	mg/l	19.4	°C	3/3/2010	14:44:34
8		9.340	mg/l	19.5	°C	3/3/2010	14:44:39
9		9.390	mg/l	19.4	°C	3/3/2010	14:44:44
10		9.390	mg/l	19.4	°C	3/3/2010	14:44:49
11		9.380	mg/l	19.4	°C	3/3/2010	14:44:54
12		9.390	mg/l	19.4	°C	3/3/2010	14:44:59
13		9.380	mg/l	19.4	°C	3/3/2010	14:45:04
14		9.350	mg/l	19.5	°C	3/3/2010	14:45:09
15		9.350	mg/l	19.4	°C	3/3/2010	14:45:14
16		9.340	mg/l	19.4	°C	3/3/2010	14:45:19
17		9.350	mg/l	19.5	°C	3/3/2010	14:45:24
18		9.350	mg/l	19.4	°C	3/3/2010	14:45:29
19		9.340	mg/l	19.4	°C	3/3/2010	14:45:34
20		9.310	mg/l	19.4	°C	3/3/2010	14:45:39
21		9.310	mg/l	19.4	°C	3/3/2010	14:45:44
22		9.300	mg/l	19.4	°C	3/3/2010	14:45:49
23		9.340	mg/l	19.4	°C	3/3/2010	14:45:54
24		9.330	mg/l	19.4	°C	3/3/2010	14:45:59
25		9.320	mg/l	19.5	°C	3/3/2010	14:46:04
26		9.330	mg/l	19.4	°C	3/3/2010	14:46:09
27		9.330	mg/l	19.4	°C	3/3/2010	14:46:14
28		9.300	mg/l	19.4	°C	3/3/2010	14:46:19
29		9.280	mg/l	19.4	°C	3/3/2010	14:46:24
30		9.290	mg/l	19.4	°C	3/3/2010	14:46:29
31		9.290	mg/l	19.4	°C	3/3/2010	14:46:34
32		9.290	mg/l	19.4	°C	3/3/2010	14:46:39
33		9.290	mg/l	19.4	°C	3/3/2010	14:46:44

Figure 3.2. The test equipment for OUR determination (b)

Procedure

Generally the test was carried out in 9 different steps in which four steps consisted of nutrient control, chemical control with 3 different concentrations starting from low concentration to high was tested. The next five measurements included sludge except two of them which only contained tap water, nutrient solution and activated sludge. Those two were taken as sludge controls. The other three had a constant volume of 100 mL of sludge and the different chemical concentrations mixed.

Each chemical was tested for its inhibitory action towards activated sludge. Different volumes of chemical and sludge were measured to know whether the chemical was toxic or not. For this, the nutrient solution which consisted of Peptone, Dextrose, Urea and K_2HPO_4 was used in the same amount in each step of test, i.e. constant volume of nutrient solution was added on control, chemical blank, chemical with activated sludge and sludge controls. The overall volume per sample was made 500 mL The measurement frequency was set to 10 seconds per measurement. Each measurement was terminated either if the oxygen level dropped below 2 mgO_2/ L or after 10 min. In the first four however the measurement was also stopped earlier if there was no significant change in the DO level.

Table 3.6 shown below was followed same for all the pharmaceuticals tested. Only the concentrations and volumes were changed for different samples. But the nutrient solution was added 8 mL for each step of test.

Table 3.6. OUR measurement Table template

S.N	Measurement	Nutrient	Chemical	Sludge
1	Control (nutrient)	16 mg/L	-	-
2	Chemical 5mg/L	16 mg/L	5mg/L	-
3	Chemical 10mg/L	16 mg/L	10mg/L	-
4	Chemical 15mg/L	16 mg/L	15mg/L	-
5	Sludge Control I	16 mg/L	-	100 mL
6	Sludge chemical 5mg/L	16 mg/L	5mg/L	100 mL
7	Sludge chemical 10mg/L	16 mg/L	10mg/L	100 mL
8	Sludge chemical 15mg/L	16 mg/L	15mg/L	100 mL
9	Sludge Control II	16 mg/L	-	100 mL

The working volume of the chemicals depends upon the standard of the stock chemical. Working volumes were prepared according to the solubility of the chemicals.

3.4.2. Mixed Liquor suspended solid (MLSS) Mixed Liquor Suspended Volatile Solid (MLVSS) method

Apparatus needed:

- Dry filters
- Tweezers
- Crucible
- Weighing balance
- Filtering device
- Measuring cylinder
- MilliQ-water
- Hot Air Oven
- Dessicator
- Pipettes

Method

The mixed liquor was collected from the test vessel after completing the measurement of DO. It was stored in each 500 mL BOD bottles and the lid was closed tightly. A dry filter paper was taken with tweezer and placed in a crucible and weighed. The filter was placed smooth down into the funnel of the filtering device.

Sample was mixed vigorously and 10 mL of the mixed liquor was taken with a pipette in one stroke. It was poured over the filter paper slowly preventing the spoilage of the liquid out of the mouth of the filtering device. Sample was filtered and cylinder was rinsed with the MQ water. The filter was removed from the device and was placed in a crucible and dried in an oven preheated at 106°C for 1 hour. The crucible was let cooled into a desiccator and weighed. MLSS was calculated as a difference between the initial mass and the current mass of the filter divided by the sample volume. This MLSS value was used later in determining OUR results

The MLSS samples were put in another oven at 550°C for an hour. After the samples had cooled down their weight was measured and the weight loss due to burning (the difference the weight before the 550°C oven and after) was calculated and divided by the sample volume of 10 mL to obtain the MLVSS. Note: MLVSS values were not used in this experiment.

Specific Oxygen Rate (SOUR) was obtained by dividing the OUR with MLSS values. This SOUR was used in calculating EC_{50} of the chemical.

3.5. Screening of Active pharmaceuticals and selection of inhibitory compounds

All above chemicals were screened for its inhibitory action by performing OUR test. Effective concentration 50 was calculated for each chemical to know the 50% inhibition of the activated sludge. The volume of above chemicals was different for some drugs because of their solubility difference.

3.5.1 Calculation of OUR and MLSS

The OUR method is already discussed above in the chapter 3.4.1. Calculation of OUR was done after taking the measurement. The temperature was maintained ±20 in all the tests.

The oxygen uptake rate is defined by the following equation:

OUR [mgO_2/L/h] = dO_2/dt (2)

The SOUR is another parameter where the OUR is related to the mass of activated sludge:

SOUR [mgO_2/L/h/mgSS] = dO_2/dt/MLSS (3)

In order to calculate the effect of chemicals the so called inhibition factor is used:

$$I = \frac{R_s - (R_{SC} - R_C)}{R_s} \quad (4)$$

In equation (4),

I =Inhibition

RS = OUR of the sample

RSC = OUR of the sample with chemical injection

RC = OUR of water with chemical injection

MLSS was calculated as

Difference between the mass of the dry filter paper and mass of the filter paper after drying in 105˚C for 1 hour divided by the sample volume of 10 mL

It can be expressed by equation (5)

$$MLSS = \frac{M1-M2}{10mL}\, g \quad (5)$$

Where,

$M1 = Mass\ of\ the\ Dry\ filter\ paper$

$M2 = Mass\ of\ the\ filter\ paper\ after\ drying$

$g = Grams$

For MLVSS calculation

$$MLVSS = \frac{M2 - M3}{10mL}\ g$$

Where,

$M2 = Mass\ of\ the\ filter\ paper\ after\ drying\ at\ 105^{\circ}C$

$M3 = Mass\ of\ the\ filter\ paper\ after\ drying$ at $550^{\circ}C$

$g = Grams$

3.5.2 Calculation of EC_{50} concentration

OUR calculation and EC_{50} concentration was calculated in the spreadsheet. At first the measurement datas of the time and the DO values were taken. Oxygen Consumption Rate by the chemical itself was decreased from OUR by chemical and sludge mixture (OUR-Chemical OUR). Then the SOUR was calculated by dividing the OUR value by MLSS value. Then the Inhibition percentage was calculated by the equation number 4.Oxygen consumption curves for each measurement were drawn and oxygen uptake rate of each sample was obtained from a slope of each linear equation. After calculating inhibition percentage, EC_{50} was calculated by plotting the graph where Concentration of chemical is plotted against the inhibition %.

4. Results and Discussions

4.1. Results of First Batch of chemicals

Out of 22 chemicals tested in the first batch, 8 chemicals were found toxic to the activated sludge. The inhibition percentage and the EC50 values are interpreted below. Aspirin, Cetrimide, Chlorpheniramine Maleate, Caffeine anhydrous, Diclofenac sodium, Ephedrine Hcl, Levamisole Hcl, and Camphor showed the toxicity towards the activated sludge during OUR measurements. The OUR measurements with their EC50 concentration are shown below in the pictures

Aspirin

Results:

Concentration (mg/L)	Inhibition %
20	17.5
15	16.8
10	11.7
EC 50	50.5 mg/l

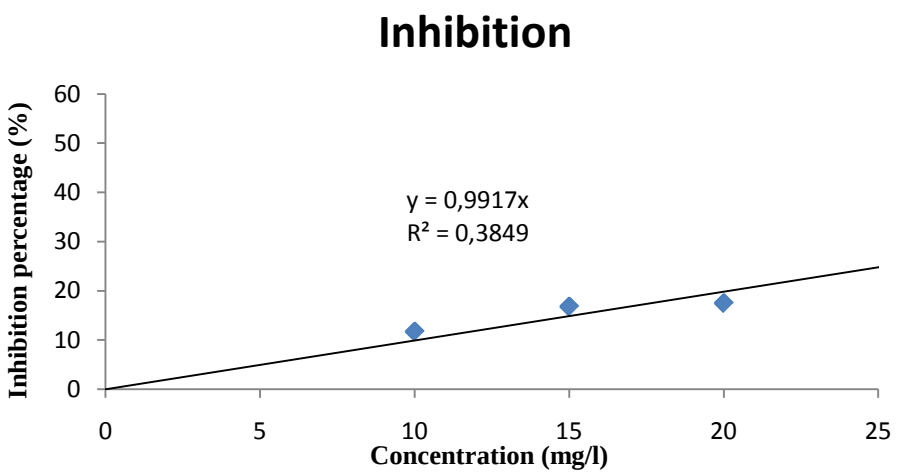


Figure 4.1. Table with different concentrations of the Aspirin with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The concentration of the Aspirin was measured with the concentrations of 20 mg/L, 15 mg/L and 10 mg/L. The EC50 value was calculated as 50.5 mg/l. The time interval was 10 minutes for each measurement. The MLSS values are shown for two sludge controls and three sludge mixed with different concentrations of the chemicals. The MLSS values for two sludge controls are nearly similar and the values for three other measurements are also nearly same. The stock Aspirin concentration was 1 g/L.

Cetrimide

Results:

Concentration(mg/L)	Inhibition %
20	58
15	22.7
10	33.2
EC 50	19.8 mg/l

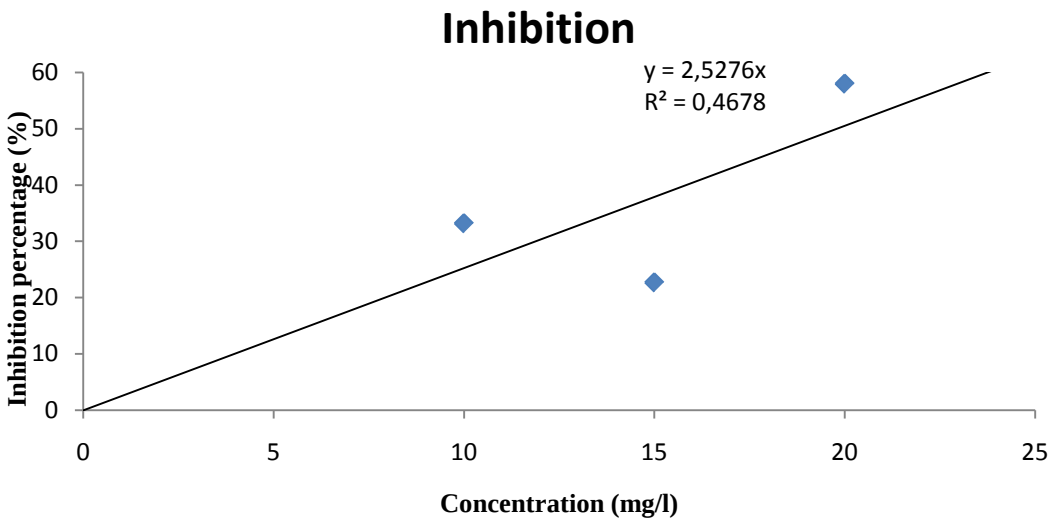


Figure 4.2. Table with different concentrations of the Cetrimide with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The inhibition was seen more with the concentration of 20 mg/L with inhibition percentage 58. The time interval was 10 minutes for each measurement. The stock Cetrimide concentration was 1 g/L. The MLSS values were nearly same for all the sludge parameters. The EC50 value obtained was 19.8 mg/l.

Chlorpheniramine Maleate

Results:

Concentration (mg/L)	Inhibition %
20	7.6
15	59.2
10	-4.2
EC 50	36.4 mg/l

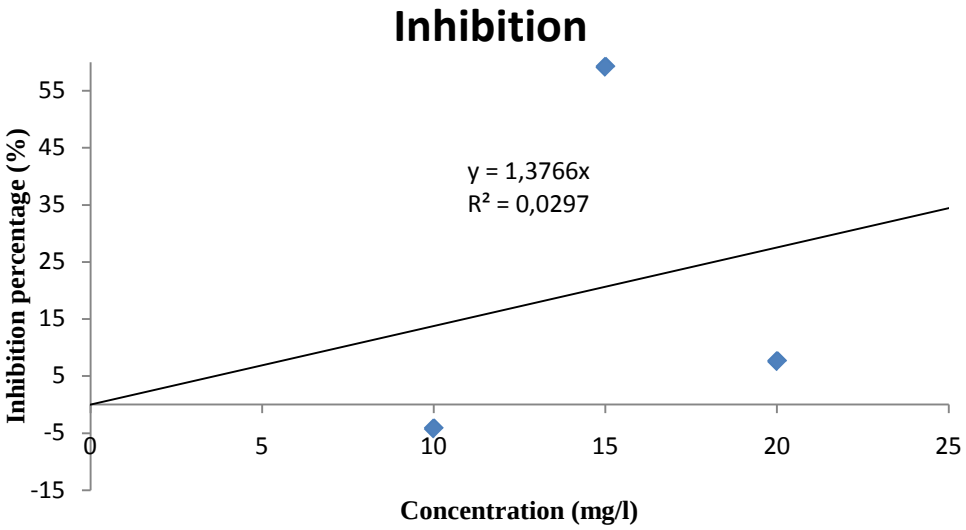


Figure 4.3. Table with different concentrations of the Chlorpheniramine Maleate with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Chlorpheniramine Maleate used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15mg/L and 10 mg/L chemicals were tested. Concentration of 15 mg/L showed the highest effect with the inhibition of 59.6 %. The EC50 was calculated as 36.4 mg/l.

Caffeine Anhydrous

Results:

Concentration (mg/L)	Inhibition %
20	54.9
15	58.6
10	47
EC 50	14.9 mg/l

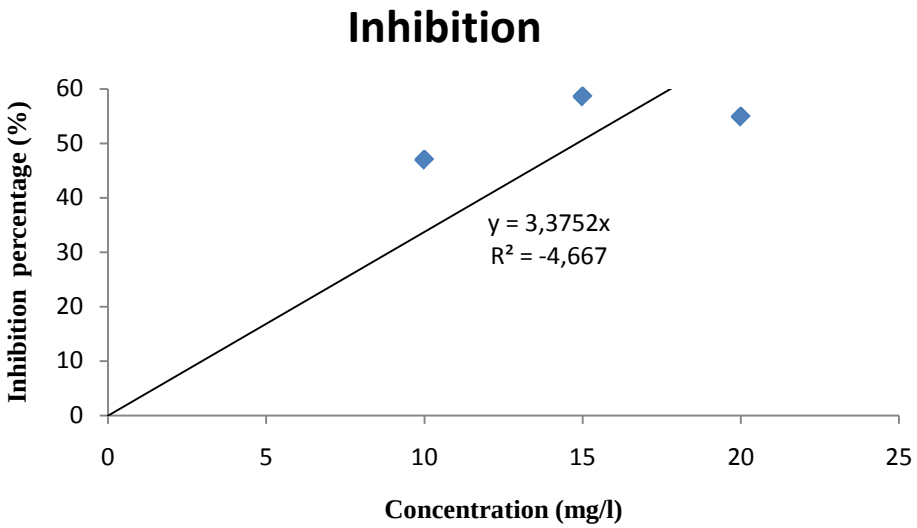


Figure 4.4. Table with different concentrations of the Caffeine Anhydrous with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Caffeine Anhydrous used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15mg/L and 10 mg/L chemicals were tested. Concentration of 15mg/L showed the highest effect with the inhibition of 58.6 %. The EC50 was calculated as 14.9 mg/l. With the concentration of 20 mg/l the inhibition was also nearly same as from 15 mg/L which was 54.9 mg/l.

Diclofenac Sodium

Results:

Concentration(mg/L)	Inhibition %
20	39.1
15	33.4
10	24.8
EC 50	23.7 mg/l

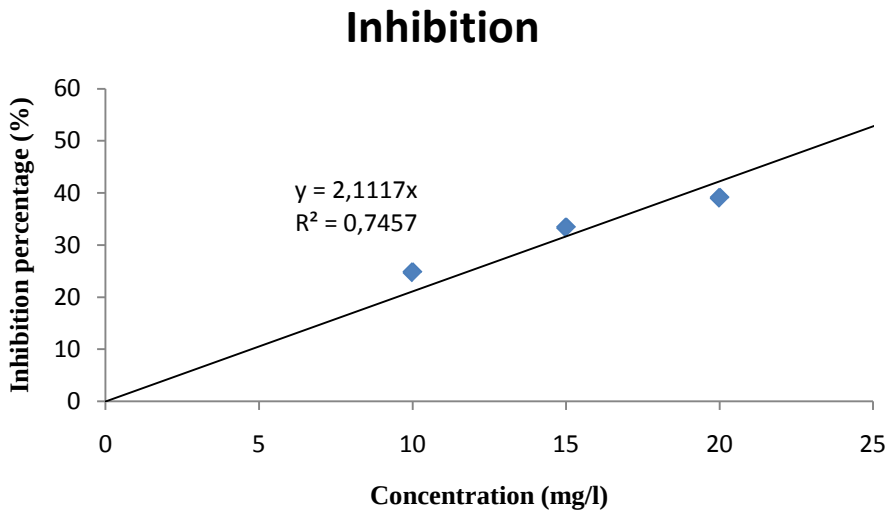


Figure 4.4. Table with different concentrations of the Diclofenac Sodium with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Diclofenac Sodium used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15 mg/L and 10 mg/L chemicals were tested. Concentration of 20mg/L showed the highest effect with the inhibition of 39.1mg/l. The EC50 was calculated as 23.7 mg/l. With the concentration of 15 mg/L and 20 mg/L the inhibition was also nearly same with little lower inhibition was seen with 10 mg/L.

Ephedrine Hcl

Results:

Concentration(mg/L)	Inhibition %
20	33.2
15	19.3
10	15.5
EC 50	32.7 mg/l

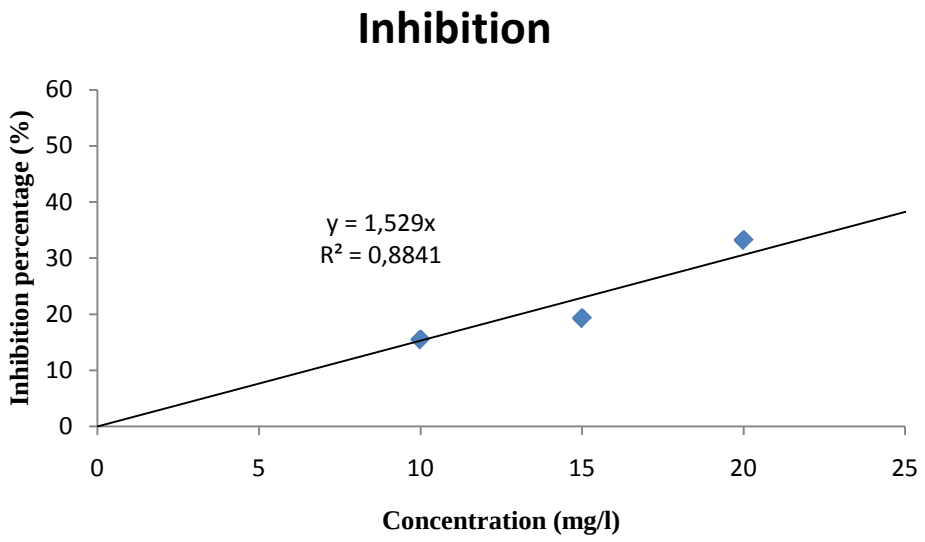


Figure 4.5. Table with different concentrations of the Ephedrine Hcl with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Ephedrine Hcl used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15 mg/L and 10 mg/L chemicals were tested. Concentration of 20 mg/L showed the highest effect with the inhibition of 33.2%. The EC50 was calculated as 32.7 mg/l. With the concentration of 15 mg/L and 10 mg/L there was lower inhibition of 19.3 and 15.5% respectively.

Levamisole Hcl

Results:

Concentration(mg/L)	Inhibition %
20	59.9
15	44.5
10	54.8
EC 50	15 mg/l

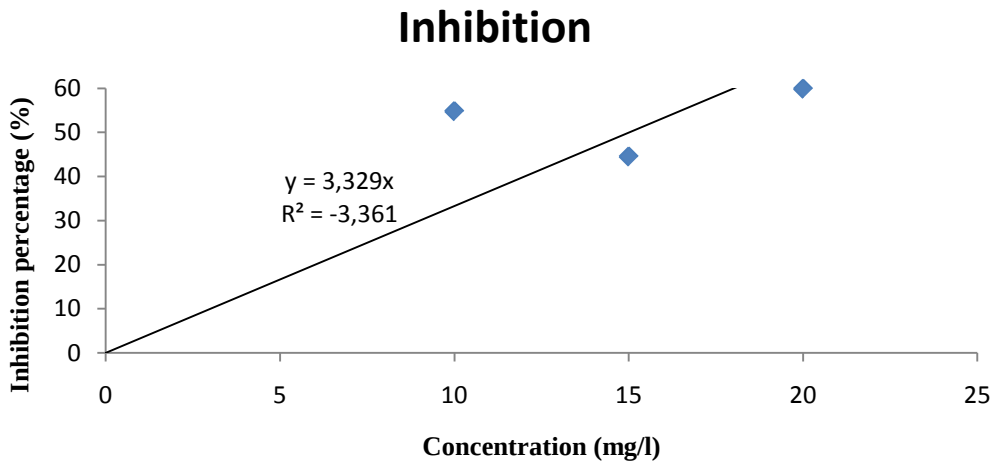


Figure 4.6. Table with different concentrations of the Levamisole Hcl with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Levamisole Hcl used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15 mg/L and 10 mg/L chemicals were tested. Concentration of 20mg/L showed the highest effect with the inhibition of 59.9%. The EC50 was calculated as 15 mg/l. With the concentration of 15 mg/L and 10 mg/L there was lower inhibition of 44.5 and 54.8 respectively.

Camphor

Results:

Concentration(mg/L)	Inhibition %
20	0
15	26
10	24.8
EC 50	56.9 mg/l

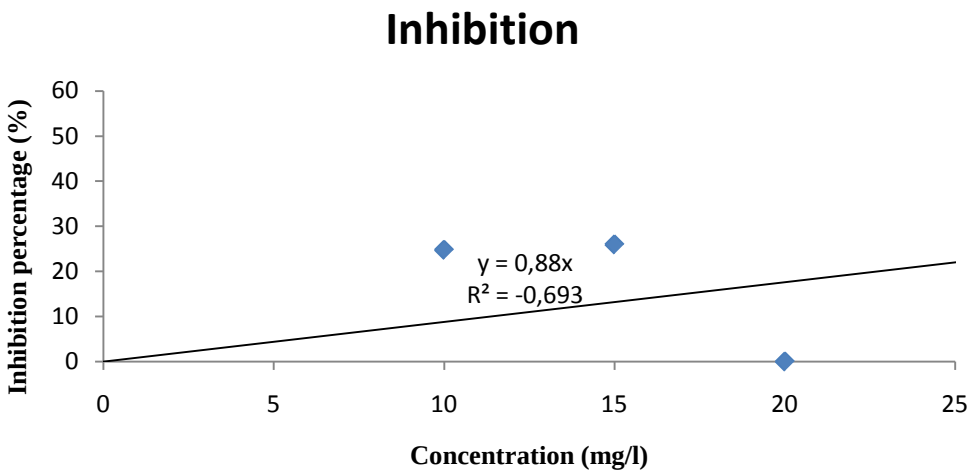


Figure 4.7. Table with different concentrations of the Camphor with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Camphor used for testing was 1 g/L. The two different concentrations of 15mg/L and 10 mg/L chemicals were tested. Concentration of 15mg/L showed the highest effect with the inhibition of 26%. The EC50 was calculated as 56.9 mg/l. With the concentration of 10 mg/L there was lower inhibition of 24.8 % which is closer to the inhibition of 15%. So there was no big difference between the inhibitions of two concentrations.

4.2. Results of Second Batch of chemicals

Out of 10 chemicals tested in the second batch, three chemicals were found toxic to the activated sludge by using OUR method. The inhibition percentage and the EC50 values of the inhibitory ones are presented below

Aminosidine

Results:

Concentration(mg/L)	Inhibition %
60	53.8
40	45.9
20	44.8
EC 50	47 mg/l

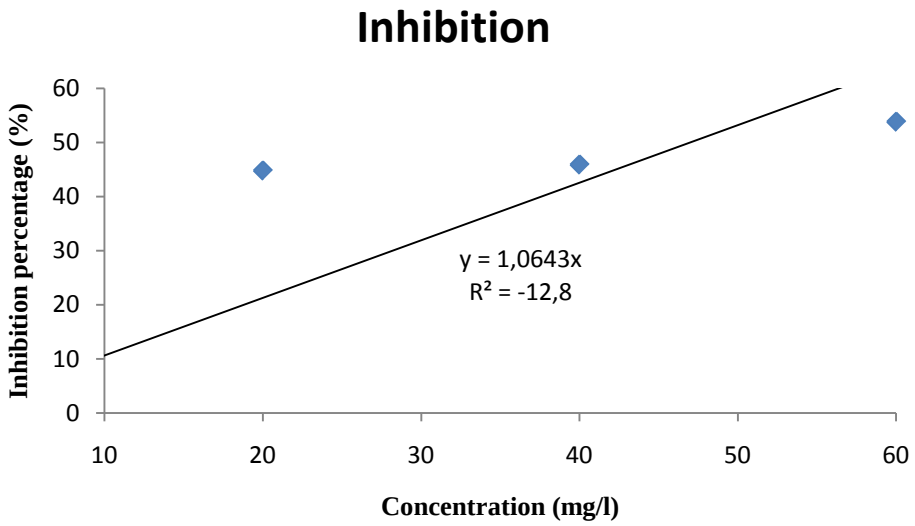


Figure 4.8. Table with different concentrations of the Aminosidine with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Aminosidine used for testing was 1 g/L. The three different concentrations of 60mg/L, 40mg/L and 200 mg/L chemicals were tested. Concentration of 60mg/L showed the highest effect with the inhibition of 53.8%. The EC50 was calculated as 47 mg/l. With the concentration of 40 mg/L and 20mg/L there was lower inhibition of 45.9 and 44.8 % respectively which are more closer to each other. So there was no big difference between the inhibitions of two concentrations. It can be seen as the three concentrations have decreasing inhibition with the decrease in concentrations.

Cetrizine

Results:

Concentration(mg/L)	Inhibition %
5	20.1
5	32
2.5	10.8
EC 50	9.8 mg/l

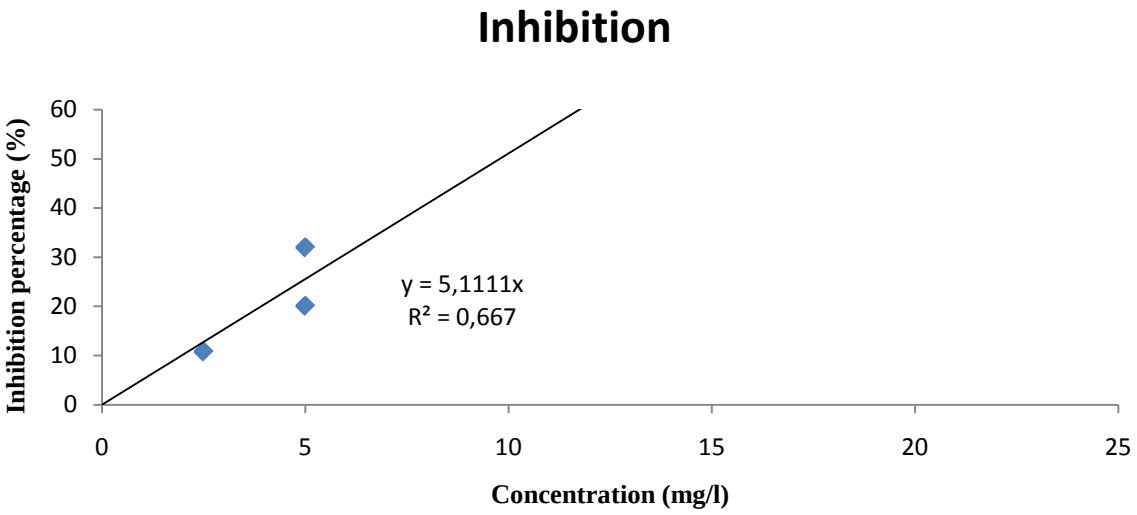


Figure4.9.Table with different concentrations of the Cetrizine with their corresponding inhibition percentage calculating EC50 and the graph representing it

The stock concentration of Cetrizine used for testing was 0.1 g/L. The three different concentrations of 5mg/L, 5mg/L and 2.5 mg/L chemicals were tested. Concentration of 5mg/L showed the high effect with the inhibition of 20.1%. Again the same concentration was used which showed the highest inhibition of 32%. The EC50 was calculated as 9.8 mg/l. With the concentration of 2.5mg/L it showed the effect of 10.8 which was nearly half inhibition than that the concentration with 5mg/L. So the result seems reasonable.

Quinine Dihydrochloride

Results:

Concentration(mg/L)	Inhibition %
5	36.3
5	39
2.5	35.9
EC 50	6 mg/l

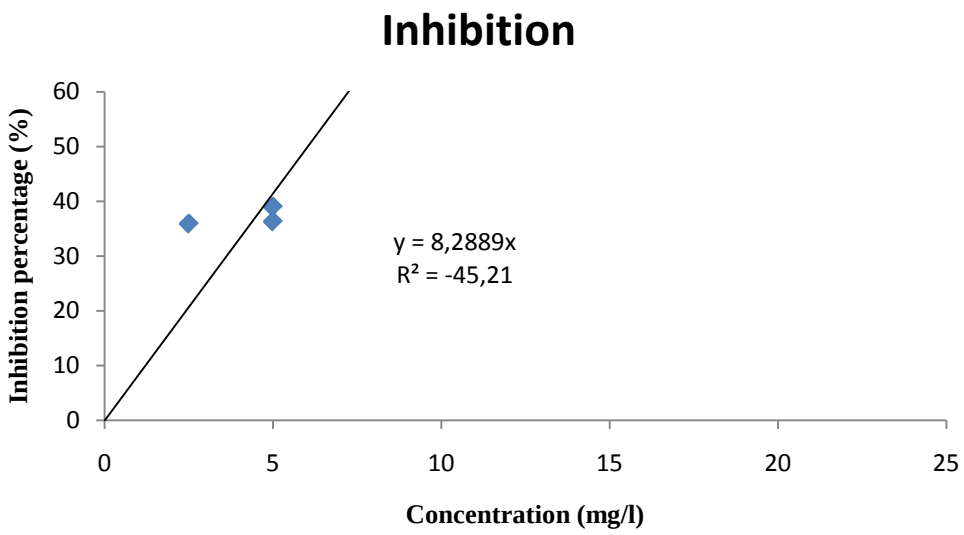


Figure 4.10. Table with different concentrations of the Quinine Dihydrochloride with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Quinine Dihydrochloride used for testing was 0.1 g/L. The three different concentrations of 5mg/L, 5mg/L and 2.5 mg/L chemicals were tested. Concentration of 5mg/L showed the high effect with the inhibition of 36.3% and again the same concentration showed 39% of inhibition. 2.5 mg/L concentration showed 35.9% of inhibition. So the result showed quite same inhibition percentage with all three concentrations. The EC50 was calculated as 6 mg/l.

With third and fourth batch of chemicals no significant toxicity was seen with OUR measurements. But few more pharmaceutical results are presented below which were non toxic to the activated sludge.

Amodiaquine Hcl

Results

Concentration	Inhibition %
60	20.3
40	29
20	17.3
EC 50	102.9 mg/l

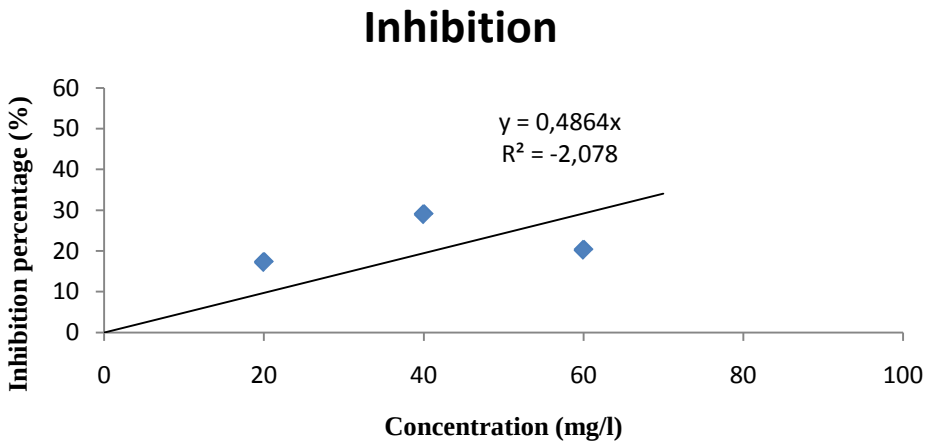


Figure 4.11. Table with different concentrations of the Amodiaquine Hcl with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Amodiaquine Hcl used for testing was 1 g/L. The three different concentrations of 60 mg/L, 40 mg/L and 20 mg/L chemicals were tested. Concentration of 40mg/L showed the high effect with the inhibition of 29% and 60 mg/L concentration showed 20.3% of inhibition. 20 mg/L concentration showed 17.3% of inhibition. So the result showed quite same inhibition percentage with all three concentrations. The EC50 was calculated as 102.9 mg/l.

Folic Acid

Results

Concentration	Inhibition %
20	18.9
15	-19.3
10	15.4
EC 50	149 mg/l

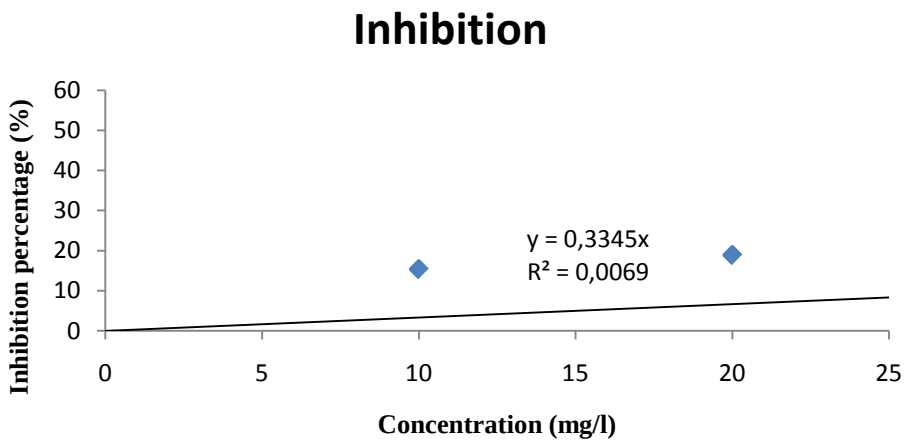


Figure 4.12. Table with different concentrations of the Folic Acid with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Folic acid used for testing was 1 g/L. The three different concentrations of 20 mg/L, 15 mg/L and 10 mg/L chemicals were tested. Concentration of 20 mg/L showed the high effect with the inhibition of 18.9% and 10 mg/L concentration showed 15.4% of inhibition. 15 mg/L concentration showed -19.3% of inhibition. So the result didn't show same inhibition percentage with all three concentrations. The EC50 was calculated as 149 mg/l.

Thiamine Hcl

Results

Concentration	Inhibition %
300	41.6
200	50.9
100	-16.8
EC 50	333.8 mg/l

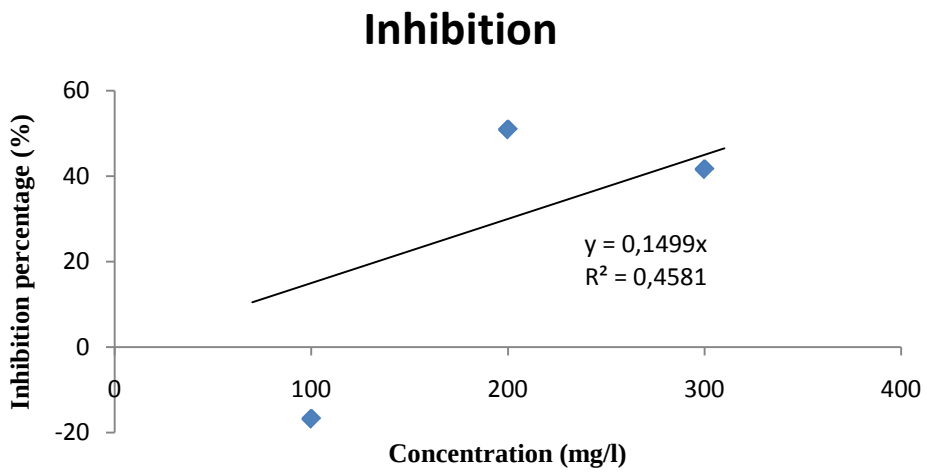


Figure 4.13. Table with different concentrations of the Thiamine Hcl with their corresponding inhibition percentage calculating EC50 and the graph representing it.

The stock concentration of Thiamine Hcl used for testing was 1 g/L. The three different concentrations of 300mg/L, 200mg/L and 100 mg/L chemicals were tested. Concentration of 200mg/L showed the high effect with the inhibition of 50.9% and 300 mg/L concentration showed 41.6% of inhibition. 100 mg/L concentration showed -16.8 % of inhibition. So the result didn't show same inhibition percentage with all three concentrations. The EC50 was calculated as 333.8 mg/l.

5. Conclusions

Different types of pharmaceuticals are manufactured in the pharmaceutical company. It is very important to do screening for these pharmaceuticals inorder to maintain the good efficiency of waste water treatment plant. There are different ways of wastewater treatment. Among them, Biological wastewater treatment has been followed inorder to remove this pharmaceuticals. Inorder to know the efficiency level of the biological treatment plant, the toxicity of these chemicals should be known.

Activated sludge is the main component of this kind of treatment plant. The sludge contains various microorganisms which consume oxygen for the respiration. When those toxic chemicals are mixed with the sludge, it decreases the oxygen consumption capacity of the microorganisms proving that the specific pharmaceutical is harmful for the biological wastewater treatment plant. This is the basic principle behind the toxicity measurement of these pharmaceuticals. The measurement technique followed was the OUR (Oxygen Uptake Rate) method. 50 Pharmaceuticals were tested with OUR method for its toxicity towards the activated sludge and 11 compounds found to be toxic with 50% inhibition at different concentrations. Drugs were tested with varying concentrations inorder to check the maximum concentration causing the toxicity. OUR was calculated as slope, SOUR was calculated by dividing OUR by MLSS values.

Effective Concentration 50 (EC50) for Diclofenac is 11.5-22.7 mg/L and the observed EC50 in this work was 23.7 mg/L. Diclofenac Sodium was found to be toxic. It is a poor biodegradable chemical. Likewise, Aspirin, Cetrimide, Cetrizine, Chlorpheniramine Maleate, Caffeine Anhydrous, Ephedrine Hcl, Levamisole Hcl, Quinine Dihydrochloride and Camphor were found toxic. These eight pharmaceuticals showed the significant inhibition of the oxygen consumption of the activated sludge tested in the laboratory. In the practical part of this work, inhibitions were calculated and graphs were plotted with concentrations vs inhibition percentage inorder to calculate the EC50 values.

Pharmaceuticals not only inhibit the oxygen consumption but also it acts as a food sometimes. When there are clumps in the sludge, the oxygen consumption rate may be affected because the bulky portions in the sludge won’t consume oxygen. Therefore the sludge should be homogenous. The main aim of this work was to find the toxic chemicals to the biological wastewater treatment plant in the pharmaceutical industry. Since the new drugs are being manufactured continuously, these drugs should be screened before they come in contact with the biological treatment system.

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